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CHARACTERISTICS OF DISPERSABLE ORGANIC COLLOIDS IN PEATS [1]

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INTRODUCTION

Although the importance of fine organic matter in soil is generally recognized, studies of soil colloids have dealt mainly with inorganic constituents. In a previous report (*16*) [3] some evidence of high base-exchange capacity of peat soils was noted. This characteristic has recently been reported by several investigators (*2, 9, 10, 11, 13*). There is also evidence that organic colloidal matter in soils possesses a large capacity for absorbing moisture (*14*).

The experiments herein reported were undertaken to determine the amount and composition of colloids in several peat-profile layers, to learn how base-exchange capacity changes during decomposition of organic materials, and to obtain information as to the nature and possible means of increasing or conserving the base-exchange capacity of soils.

PREVIOUS WORK

The base-exchange property of soils was reported by Way in 1850 (*30*). It was recognized by Gedroiz (*7*) and by Odén (*14*) that organic as well as inorganic colloidal matter in soils may contribute to their base-exchange values. In an earlier study the writer (*17*) found evidence of a large base capacity in peat soils. However, in the more acid peats, a large part of the total base-exchange capacity was due to exchangeable hydrogen. Soon after the publication of this report a study by Hissink (*9*) with some 13 peaty soils came to the writer's attention. His conclusions supported this view. Alben (*1*) reported finding the base-exchange capacity of peat to be some seven times that of normal soil. Baver (*2*) studied the effect of removing organic matter from 4 soils by hydrogen-peroxide treatment, and concluded that from 30 to 60 per cent of their base-exchange capacity was due to organic matter. McGeorge (*11*) worked with a score of soils, most of which were of a peaty character, and obtained evidence that high base-exchange capacity was related to the lignin content.

Recently Smolik (*24*) reported base replacement far greater for soils rich in organic matter and markedly reduced by treatment with

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[3] Reference is made by number (italic) to Literature Cited, p. 110.

hydrogen peroxide. Odén (*14*) about a decade ago reported that humic-acid forms a salt with ammonia and that liming results in the formation of a calcium humate which he regarded as an excellent regulator or buffer for the prevention of a strong acid reaction. It was of low solubility and difficult to wash out. Demolon and Barbier (*3*) were also led by their experiments to suggest that the clay and humate fractions form a complex, and that this argillo-humate complex is influenced by the cations absorbed. Doughty (*4*) studied phosphate fixation in peat, and concluded that calcium, iron, or aluminum when present in soluble form cause precipitation of phosphate in peat. Similar results were obtained by Marshall (*12*), who reported that calcium humate exercises a protective action and sensitizes the soil colloid. Dunnewald (*5*) noted the relation of calcium carbonate to soil organic-matter content and vegetation. Recently Mattson (*13*) reported investigations of base capacity of iron and aluminum humates when in different proportions and also at different pH values. High humic content in association with an alkaline reaction seems to possess large base-absorbing capacity.

A review of the literature indicates the need of further information in regard to the characteristics of natural organic soil colloids in order that methods may be developed of increasing and maintaining high base-exchange capacity in soils. In the present study natural organic soil colloids from several peat profiles have been isolated and studied, perhaps for the first time.

EXPERIMENTS WITH PEAT COLLOIDS

DESCRIPTION OF PEAT-PROFILE SAMPLES

Profile samples of representative peat formations from widely separated sections were secured for the present study and are briefly described as follows:

1. Saw-grass peat from the Brown plantation, Belle Glade, Fla., described in a previous publication of this department (*6*).
2. Saw-grass peat, fresh sample, taken in slightly shallower peat, about one-half mile distant from No. 1.
3. Virgin willow-sedge peat from Lake Labish, near Salem, Oreg.:
   - 0 to 6 inches——Dark-brown finely fibrous woody-sedge peat.
   - 6 to 48 inches——Brown fibrous peat of sedge and soft wood.
   - 48 to 60 inches——Fibrous peat with some sedimentary materials, diatoms, sponge spicules, spores, and millet fragments.
4. Similar to No. 3 and about one-fourth mile distant. Cropped about 20 years.
5. Sphagnum peat from Cottage Lake, near Seattle, Wash.
   - 0 to 2 inches——Gray-brown sphagnum moss with related vegetation, such as swamp laurel, wild cranberry, and rhododendron.
   - 2 to 10 inches——Raw yellow-brown sphagnum peat.
   - 12 to 24 inches——Brown fibrous sphagnum peat.
   - 24 to 36 inches——Dark-brown fibrous peat, partly decomposed.
6. Clackamas peaty gravelly loam, or "loose land" from West Stayton, near Salem, Oreg.
   - 0 to 8 inches——Brown sooty gravelly loam.
   - 18 to 30 inches——Yellowish-brown gravelly loam.
7. Sedimentary tule-sedge peat, Lower Klamath Marsh, Oreg.
   - 0 to 14 inches——Dark-brown fibrous tule-sedge peat with some carbonized material.
   - 14 to 28 inches——Gray-brown finely fibrous peat.
   - 28 to 42 inches——Gray sedimentary muck with diatomaceous material.

8. Sedimentary peat, Orleans County, N. Y., collected and described by A. P. Dachnowski-Stokes. A typical profile of peat areas in the western Ontario glacial-lake plain.
   0 to 6 inches.....Woody peat.
   12 to 18 inches.....Sedimentary peat.
   30 to 36 inches.....Reed peat.
   60 to 72 inches.....Sedimentary peat.

EXPERIMENTAL METHODS

The method of separation of organic colloid was similar to that described by Robinson and Holmes (*21*) with modifications, as noted later. Absorption tests were made according to the method of Robinson (*19*), and the general inorganic analytical procedure followed was that of Robinson (*18*). Base-exchange capacity was determined by treating with one-twentieth normal hydrochloric acid, then saturating with normal neutral barium chloride, and, after washing out the excess of saturant with warm distilled water till free of chlorides, displacing with normal ammonium chloride. The barium absorbed was determined gravimetrically as barium sulphate.

Organic analyses were made according to the method of Waksman and Stevens (*29*) as modified by Feustel and Byers (*6*). Lignin and hemicellulose were obtained from flax shives and sphagnum moss by the method of Phillips (*15*).

PRELIMINARY EXPERIMENTS

Some preliminary tests were made to determine the most suitable procedure for separating the colloid from peat samples. These included the use of different amounts of sample, removal of bases, hand kneading, electric agitation (*21*), mechanical shaking, agitating by means of a Bouyoucos shaker, addition of sodium oxalate, and previous extraction with ether. The effects of amount of dilution (*22*), rate of centrifuging, and yield and character of different fractions were also considered.

From these tests it was found advisable to use sufficient fresh moist peat to yield from 100 to 200 gm. of material that would pass through a 2-mm. sieve. Shaking the sample overnight in a 1-gallon sirup bottle two-thirds full of distilled water, with a slight addition of sodium oxalate (approximately 0.1 gm.), proved very helpful. The shaker used for preparing samples for mechanical analysis was fitted with a drawer to hold two 1-gallon bottles. The electric agitator was used before each run of the supercentrifuge, and material coarser than 2 mm. was omitted after the second run. Eight runs were made, in which a total of 150 to 200 liters of water was used, according to the profile dealt with. Running the peat suspension through the centrifuge at the rate of 1 liter in 5 seconds separated a colloid fraction mainly below 1μ, with a few aggregates in excess of 2μ in diameter. Satisfactory yields of colloid were thus secured.

This colloidal fraction, reduced by a battery of Pasteur-Chamberland filters to slightly less than 2 quarts and homogenized by passing through a fine Gooch crucible, carried sufficient solids to give a 2 or 3 gm. sample from 200 c. c. of suspension. Most of the samples employed for base capacity and analyses weighed approximately 2 gm.

The bowl fraction and even material between 1 and 2 mm. in diameter were found to manifest somewhat colloidal properties.

AMOUNT AND ABSORPTIVENESS OF COLLOIDS FROM PEAT

The amount (yield) and absorptiveness (character) of peat colloids are shown in Table 1.

TABLE 1.—*Amount and absorptiveness of peatcolloids*

Sample No.	Location	Description of sample	Depth	pH	Dry weight of sample	Material greater than 2 mm. in diameter	Material less than 2 mm. in diameter	Weight of dry colloid extracted	Colloid present in 2-mm. fraction	Ash in colloid	Absorption of water over 3.3 per cent H_2SO_4
			In.		*Gms.*	*Gms.*	*Gms.*	*Gms.*	*P. ct.*	*P. ct.*	*P. ct.*
1	Belle Glade, Fla.	Saw-grass peat	0–4	[a]5.3	94.75	14.1	80.65	2.886	3.05	18.28	------
			4–6	6.2	26.79	4.1	22.71	1.432	3.34	18.60	------
			32	6.3	29.21	2.3	26.95	1.933	6.62	26.66	------
			49	6.3	22.40	5.2	17.16	2.088	9.32	------	------
			63	6.7	21.19	.8	20.43	3.075	14.51	------	------
			94–96	7.1	20.60	3.1	17.54	1.980	9.61	21.70	------
2	Do	do	0–4	4.6	236.10	133.0	113.10	20.100	25.70	25.70	46.86
			4–6	5.2	142.40	32.0	110.40	26.600	24.10	24.10	47.13
			32	6.6	129.70	15.0	114.70	45.100	39.30	39.30	43.61
			49	6.6	115.80	37.0	78.80	20.860	26.40	26.40	43.86
			63	6.8	104.60	31.0	73.60	22.000	29.90	29.90	43.66
			86–88	7.2	114.70	32.0	82.70	46.800	56.50	56.50	39.50
3	Salem, Oreg.	Virgin willow-sedge peat.	0–6	6.5	256.30	10.2	246.00	54.970	22.34	62.59	33.34
			12–42	6.5	116.20	3.8	112.40	55.130	31.25	61.49	38.56
			48–60	6.0	111.30	2.8	108.50	56.740	52.30	------	46.67
4	Do	Willow-sedge peat (cropped 20 years).	0–6	6.4	240.00	8.4	231.60	32.040	13.83	48.21	39.23
			12–42	6.5	102.90	2.5	100.70	26.440	26.25	28.96	38.52
			48–60	7.1	102.30	1.5	100.80	22.780	22.59	32.94	35.77
5	Seattle, Wash.	Sphagnum peat	2–10	5.1	652.00	300.0	352.00	18.500	5.28	19.59	45.57
			12–24	6.4	80.60	40.0	40.60	2.178	5.35	16.66	46.51
			24–36	6.4	311.00	200.0	111.00	14.430	13.00	11.71	42.16
6	Salem, Oreg.	Clackamas peaty gravelly loam (loose land).	0–8	5.7	1,142.00	50.0	1,092.00	115.400	10.57	53.52	37.24
			18–30	6.0	93.00	40.0	530.00	26.100	4.92	66.48	29.23
7	Lower Klamath Marsh, Oreg.	Sedimentary tule-sedge peat.	0–14	7.1	176.00	1.0	177.00	53.28	30.10	83.19	34.74
			14–28	7.0	181.00	0	181.00	18.28	10.10	63.83	33.58
			28–42	6.2	186.60	0	186.60	8.14	4.35	56.77	50.83
8	Orleans County, N. Y.	Semidentary peat	0–6	5.8	164.00	25.0	139.00	11.90	8.56	53.57	42.01
			12–18	5.8	121.00	15.0	106.00	16.65	15.71	18.92	46.04
			30–36	5.7	80.00	1.0	79.00	11.74	14.86	13.04	45.79
			60–72	7.4	60.60	.5	60.10	9.18	15.30	20.37	50.16

[a] See U. S. Dept. Agr. Tech. Bul. 214 (*6*), for pH data for Florida peat.

The peat samples ranged from neutral to distinctly acid in character. Moist samples weighing from 200 to 1,000 gm. were used, the inititial moisture content ranging from 100 to 800 per cent. Considerable difference was shown in the content of coarse material in the different layers of a single profile. The sedimentary layers were low in ash and yielded relatively large percentages of organic colloid. This colloid yield ranged from 3.05 to more than 56.5 per cent of the sample passing a 2-mm. sieve, expressed on a dry-weight basis.

Absorption of moisture over 3.3 per cent sulphuric acid was determined with samples which had been slowly dried on the edge of the steam bath and ground to pass through 130-mesh bolting cloth. The average absorption of peat colloid was 45 per cent as compared to about 30 per cent for inorganic soil colloid. The values obtained ranged from approximately 29 to 50 per cent. The lower values are for colloids having large ash content.

PROXIMATE ORGANIC COMPOSITION AND BASE-EXCHANGE CAPACITY OF PEAT COLLOIDS

Organic analyses were made of the different layers in peat profiles. These are summarized in Table 2.

TABLE 2.—*Proximate organic composition of peat colloids*

[In per cent of colloid]

Sample No.	Location	Description of sample	Depth	Dry weight of colloid used	Ether-soluble material in colloid	Alcohol-soluble material in colloid	Hot-water soluble material in colloid	
							Dry matter	Ash
			Inches	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Belle Glade, Fla.	Saw-grass peat	0-4	3.88	0.206	0.42	4.81	0.87
			4-6	3.04	.336	.46	2.78	.68
			32	4.11	.224	.27	1.20	.39
			49	2.98	.269	.58	1.53	.57
			63	2.20	.432	.61	1.55	.72
			86-88	3.13	.517	.58	1.43	.55
3	Salem, Oreg.	Virgin willow-sedge peat.	0-6	5.99	.250	.86	3.33	.56
			12-42	4.29	.110	.67	1.79	.38
			48-60	6.94	.870	.50	1.22	.22
4	Do.	Willow-sedge peat (cropped 20 years).	0-6	3.10	.340	.50	3.07	.37
			12-42	3.46	.220	.87	1.96	.42
			48-60	3.33	.420	.71	1.63	.47
5	Seattle, Wash.	Sphagnum peat	2-10	1.48	.550	4.30	7.79	.....
			12-24	.30	.500	5.33	7.43	.....
			24-36	1.11	1.160	6.23	7.29	.....
6	Salem, Oreg.	Clackamas peaty gravelly loam (loose land).	0-8	9.23	.130	.57	1.15	.....
			18-30	5.22	.140	.38	1.00	.....
8	Orleans County, N. Y.	Sedimentary peat	0-6	1.40	1.250	2.16	5.75	1.10
			12-18	1.48	.540	2.12	6.21	1.28
			30-36	1.61	.600	1.22	3.68	.58
			60-72	1.08	.910	2.08	5.00	1.00

Sample No.	Location	Hemicellulose	Cellulose	Lignin			Base-exchange capacity per gram of colloid	Total nitrogen	
				Gross lignin	Ash	Ash-free lignin [a]		In whole peat	In colloid fraction
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Milliequivalent*	*Per cent*	*Per cent*
1	Belle Glade, Fla.	3.78	0.73	44.25	0.77	43.48	0.695	3.58	3.54
		6.24	.92	51.30	.91	50.39	.711	3.02	3.54
		2.53	1.32	75.22	.72	74.59	.870	3.14	3.58
		1.53	3.26	74.91	.65	74.26	.664	2.63	3.16
		3.23	3.11	72.75	.83	71.92	.729	2.81	3.35
		5.61	.95	59.92	2.96	56.96	.605	2.09	1.81
3	Salem, Oreg.	4.17	2.08	55.75	29.42	26.33	.338	2.43	5.66
		3.98	1.93	54.06	22.35	31.71	.384	2.01	4.02
		1.83	3.05	58.06	34.22	23.84	.437	1.83	4.72
4	Do.	5.93	1.49	49.73	27.44	22.29	.405	2.34	3.22
		4.61	2.45	58.25	12.64	45.61	.374	2.43	3.52
		4.43	2.22	62.84	12.73	50.11	.326	2.48	5.81
5	Seattle, Wash.	29.91	14.59	29.74	5.67	24.07	.850	.95	3.00
		32.23	10.90	27.03	4.53	22.50	.729	1.42	3.83
		29.95	7.66	46.54	5.28	41.26	.491	1.56	2.55
6	Salem, Oreg.	3.41	2.75	34.43	20.01	14.42	.165	.76	1.70
		3.57	4.77	11.73	8.54	3.19	.086	.32	.50
8	Orleans County, N. Y.	3.60	2.83	40.44	15.47	24.97	.533	2.64	2.02
		4.86	4.86	54.42	4.29	50.13	.613	2.63	2.74
		5.31	5.39	64.83	2.83	62.00	.596	2.20	3.51
		8.70	5.00	54.00	5.08	48.92	1.218	2.05	3.81

[a] Estimated by difference.

The method used seems to yield more satisfactory results with material low in inorganic matter. The hemicellulose content of sphagnum or high-moor peat appears to be relatively large. Of special interest is the so-called lignin content, which constitutes more than half of the sample taken in several cases, when expressed on the ash-free basis. The saw-grass peat carries a large amount of ligneous material, and profile No. 6 is especially well supplied. Old, sedi-

mentary layers of low ash content appear to run high in ligneous material.

The base-exchange capacity is expressed in milliequivalents per gram of colloid. There appears to be a tendency for a high base capacity to correlate with high content of ligneous substance; further evidence of this will be presented later. The state of this material as conditioned by the presence of mineral matter and bases may affect this value, as suggested by data such as are shown for the sixth-foot layer of peat sample No. 8.

Total nitrogen was determined for the peat and also for its colloid fraction. The nitrogen content of the colloid is usually higher, and in some soils, as in Nos. 3 and 4, there is approximately twice as much nitrogen in the colloid as in the whole peat. Determinations of ammonia nitrogen in colloid samples from the three layers of profile No. 3 were made by distillation with magnesium oxide to determine whether much exchange ammonia was present. The values obtained for the three layers, beginning with the top one, were 0.065, 0.072, and 0.061 per cent ammonia nitrogen. This is only a small fraction of the total nitrogen and does not account for the concentration of nitrogenous matter in the colloid part of the peat. It appears that two decades of cropping have materially lowered the nitrogen content of willow-sedge peat. This large amount of nitrogen, if available, would enhance the value of colloidal organic matter in relation to soil productiveness. The content of nitrogen in sphagnum peat colloid is low compared to that of the sedge, or low-moor, peat colloid.

BASE-EXCHANGE CAPACITY OF DIFFERENT PEATY MATERIALS

The amounts of colloid secured from certain soils were not adequate for full analyses, yet the base-exchange capacities were determined and are given in Table 3.

TABLE 3.—*Base-exchange capacity of various peaty materials*

Sample	Description of sample	Depth	Description of material	Base-exchange capacity per gram of colloid
		Inches		*Milliequivalents*
A	Saw-grass peat from profile No. 1	0–4	Natural peat	0.378
			Fraction 1 to 2 mm. diameter wet	.361
			Fraction 1 to 2 mm. diameter dry	.293
			Fraction 1 to 2 mm. diameter dry, ground.	.376
			Centrifuge bowl fraction	.509
Do	do	4–6	do	.664
B	Colloids in saw-grass peat from profile No. 1.	0–4	do	.464
		4–6		.950
		32		1.019
		49		.469
		63		.558
		96		.962
C	Colloids in tule-sedge peat, Klamath, Oreg.	0–14		.265
		14–28		.127
		28–42		.255
D	Dopplerite[a] from the Netherlands			.736

[a] Supplied by A. P. Dachnowski-Stokes.

The base-exchange capacity appears to increase with fineness of natural sample and with grinding, and to be reduced by drying. The highest base-exchange value for saw-grass peat colloid was obtained from the sedimentary layer at a depth of 32 inches. Colloid from Klamath peat contains a large portion of diatomaceous (siliceous) matter and is of only medium base capacity. The layer of saw-grass peat taken at a depth of 32 inches is sedimentary. The high base capacity obtained for such mucklike material may be due in part to molecular concentration at the interfaces of the mixture. The indicated tendency is for dilution with sand to increase the base capacity per gram of colloid.

The dopplerite is found in veins or layers in peat, where it has presumably accumulated over a long period of time (*27*). The sample was jet black, highly colloidal, and had a moisture content of 688 per cent when received. The base-exchange capacity for this substance is large.

EXPERIMENTS WITH COMPOSTED MATERIALS

LOSS OF DRY ORGANIC MATTER FROM COMPOSTED PLANT MATERIALS

In order to provide material for the study of base-exchange capacity of decaying organic matter at different stages of decomposition, several dozen jars were arranged for composting in the soil bacteriology greenhouse at Arlington Experiment Farm, Rossyln, Va. Duplicate series of stoneware jars were employed for each of the organic materials used. These materials were sweetclover straw, flax shives, sphagnum moss, sphagnum peat, and saw-grass peat. Chemical or other treatments made to ascertain their effect on decomposition and base-exchange capacity are indicated in Tables 4 and 5. Observations were made periodically to determine rate of carbon-dioxide evolution, temperature reaction, and moisture content. Watering, except for water-logged jars, was usually just sufficient to maintain a little free liquid which was drained out by decantation weekly and returned to the top of the material in the jar. Carbon-dioxide measurements were for one pair of jars of each series, for only one hour a week, and by the method of Humfeld (*10*).

The loss in organic materials is shown in Table 4.

TABLE 4.—*Loss in organic materials composted four months*

[Loss on dry-matter basis]

Treatment	Sweetclover straw		Flax shives		Sphagnum peat		Sphagnum moss	
	Loss	Residue	Loss	Residue	Loss	Residue	Loss	Residue
	Per cent	*pH*	*Per cent*	*pH*	*Per cent*	*pH*	*Per cent*	*pH*
Distilled water	53.9	7.7	11.3	5.9	0.0	4.2	8.0	4.8
Soil and manure infusion	50.4	7.8	11.5	6.0	6.4	4.2	8.5	4.3
3 per cent $CaCO_3$	54.0	8.0	12.6	6.6	3.1	5.2	[a]9.0	4.8
3 per cent $CaCO_3$ and 2 per cent NH_4NO_3	55.7	8.2	27.0	6.7	10.9	5.6	.....	.....
3 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4	57.5	8.0	29.5	7.1	12.0	4.5	18.2	4.7
2 per cent NH_4NO_3 and 2 per cent K_2HPO_4[b]	48.3	8.1	28.7	5.8	10.6	4.8	.....	.....
3 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4 (water-logged)	47.5	8.1	25.3	7.2	10.0	4.2	14.0	4.6

[a] 10 per cent sweetclover added instead of $CaCO_3$.
[b] 3 per cent $CaCO_3$ added at end of 60 days.

The sweetclover straw underwent a flash decomposition during the first six weeks, as evidenced by temperature rise and carbon dioxide evolved. Thermograph records show that the mean temperature of the greenhouse air was approximately 24° C. During the first five weeks sweetclover composts ran temperature 4° or 5° above that of the air. Insulation of two flax jars to conserve heat of reaction appeared to aid decomposition. The sweetclover composts lost from 47 to 60 per cent of the original organic matter in four months. Decomposition of other materials was slow and ranged from 6 to 30 per cent. The flax had already undergone flash decomposition in retting and was also oily. The acid sphagnum peat, and the saw-grass peat also, proved to be resistant to decay.

The sphagnum moss seemed to undergo more active decomposition than did the older sphagnum peat. Sphagnum decomposition appears to have been aided by nitrate additions.

TABLE 5.—*Base-exchange (milliequivalents) capacity of plant materials and composts*

Material	Treatment	Base-exchange capacity per gram of material		
		Fresh material	Material composted 2 months	Material composted 4 months
Sweetclover straw (3-gallon jars).	None	0.125		
	Soil infusion		0.211	0.168
	5 per cent $CaCO_3$		.188	.323
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4.		.212	.291
	2 per cent NH_4NO_3 and 2 per cent K_2HPO_4.[a]		.154	.223
	Distilled water only		.217	.202
Sphagnum moss, 0–2 inches (2-gallon jars).	None	.490		
	Soil infusion		.528	.569
	10 per cent of sweetclover added		.508	.596
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4.		.495	.570
	Same as above, with high water table		.507	.615
	Distilled water		.451	.589
Sphagnum moss, 2–10 inches (2-gallon jars).	None	.570		
	Soil infusion		.559	.631
	5 per cent $CaCO_3$		.504	.591
	5 per cent $CaCO_3$ and 2 per cent NH_4NO_3		.492	.576
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4.		.520	.588
	2 per cent NH_4NO_3 and 2 per cent K_2HPO_4.[a]		.497	.619
	Distilled water		.500	.662
Flax shives (1-gallon jars)	None	.067		
	Soil infusion		.073	.168
	5 per cent $CaCO_3$		.094	.185
	5 per cent $CaCO_3$ and 2 per cent NH_4NO_3		.086	.161
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4.		.090	.164
	2 per cent NH_4NO_3 and 2 per cent K_2HPO_4.[a]		.080	.214
	Distilled water		.073	.139
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4.[b]			
Saw-grass peat, 0–4 inches (1-gallon jars).	None	.695		
	Soil infusion		.598	.689
	5 per cent $CaCO_3$ and 2 per cent NH_4NO_3		.565	.581
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4, and well drained.		.577	.667
	Distilled water		.567	.677
Wheat straw (350-pound lots).	None	.209	(c)	
	Water		.219	
	300 pounds acid peat added		.193	
	Chopped, 14 pounds calurea containing 37 per cent N added.		.206	
	Long, 14 pounds calurea added		.202	
	Chopped, 42 pounds dried blood added		.230	
	Long, 42 pounds dried blood added		.168	

[a] 5 per cent $CaCO_3$ added at end of 60 days.
[b] Jars insulated for control of heat.
[c] Composted six weeks.

Four 1-gallon composts of saw-grass peat were provided for each of three layer samples. This material decays very slowly. Neither inoculation nor drainage gave significant results. Addition of nutrients appeared to increase the rate of decomposition during the first two months. Decomposition was most active with soil from the layer 0 to 4 inches from the surface, and losses of dry organic matter up to approximately 20 per cent were indicated. The material from the layer between depths of 4 and 6 inches lost about half as much as the fresher surface material, while the older material from the layer at a depth of 32 inches gave values that were scarcely significant.

Composite samples were taken at the end of two months and again after four months of composting. These were weighed, dried, ground, and subjected to base-capacity tests as summarized in Table 5.

The initial plant materials used in composts were found to manifest base-exchange capacities in different degrees, which might be of importance in choosing material for green manure or stable litter. Base-exchange capacity appears to increase during decomposition. Chemicals that aid decomposition seem to favor increase in base capacity, especially during the first half of the decomposition period. They may affect reaction, aid formation of additional products, or affect the physical state of the system.

Samples of composts of wheat straw prepared for mushroom growing were supplied by Edmund B. Lambert, of the Bureau of Plant Industry, who suggested that chopping helps in decomposition by aiding compaction or perhaps by exposing cut ends to attacks of decomposition microorganisms. Base-exchange capacity tests indicate that chopping is of value in promoting decomposition.

A study of these materials and certain of the compost residues is reported in Table 6.

TABLE 6.—*Proximate composition (per cent) of plant materials and compost residues* [a]

Material	Treatment	Ether soluble	Alcohol soluble	Hot-water soluble	Hot-water soluble ash	Hemicellulose	Cellulose	Lignin	Lignin ash
Sweetclover straw	None, dry	0.90	4.89	8.96	1.56	8.26	18.67	15.68	0.51
	Distilled water	1.02	3.60	7.17	1.93	14.74	17.10	26.98	1.07
	5 per cent $CaCO_3$	.49	3.12	8.06	.30	10.62	3.94	43.98	5.69
	5 per cent $CaCO_3$ and NH_4NO_3	1.36	3.31	9.43	2.57	11.27	10.91	31.32	8.97
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4	.87	2.78	12.14	4.92	9.92	10.23	25.82	6.35
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4, colloidal fraction	.48	2.05	21.52	6.34	6.26	11.87	27.76	6.35
Flax shives	None, dry	.69	2.66	3.38	.30	8.31	24.20	25.06	.43
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4	1.09	2.10	6.38	1.68	23.94	5.17	51.47	1.18
Sphagnum moss, 0–2 inch layer	None, dry	1.62	3.22	4.54	.43	12.94	13.61	18.77	1.28
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4	.42	5.43	6.96	2.07	20.20	6.07	44.39	2.65
Sphagnum peat, 2–10 inch layer	None, dry	.72	3.14	4.87	.06	11.11	9.50	22.51	1.69
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4	1.33	5.58	7.14	2.40	23.31	13.27	28.25	1.20
Saw-grass peat, 0-4 inch layer	None, dry	.76	2.25	7.16	1.92	7.78	5.50	43.91	1.61
	5 per cent $CaCO_3$, 2 per cent NH_4NO_3, and 2 per cent K_2HPO_4, with drainage	1.58	4.23	7.75	2.71	25.94	3.42	50.57	3.09
Wheat straw	None, dry	1.36	4.90	7.21	.....	25.89	38.97	13.33	1.18

[a] Composted 4 months.

PROXIMATE ORGANIC COMPOSITION OF PLANT MATERIALS AND COMPOSTS

Organic analyses were made of plant materials used in composts as well as of numerous composted materials. The results are presented in Table 6. The decrease in more soluble and less resistant constituents is partly masked by additions of soluble nutrients. Except in the case of certain saw-grass peat composts, drainage was not provided. The most significant change in composition is the concentration of ligneous material. This tends to parallel the increase in base-exchange capacity as decomposition progresses. (Table 5.)

Determinations of total nitrogen were made for several samples. Threshed sweetclover straw was found to contain 1.223 per cent nitrogen. After the sweetclover had been composted with distilled water for four months the nitrogen content of the residue was found to be 2.44 per cent. No loss of nitrogen is indicated, as the residual dry organic matter was 47.8 per cent of the initial sample. Sphagnum moss contained 1.68 per cent nitrogen, and after being composted with distilled water for four months the content of the residue, as determined, was still 1.68 per cent nitrogen, indicating a loss of nitrogen proportional to dry matter.

EXPERIMENTS WITH FRACTIONATED SUBSTANCES

STUDIES WITH CERTAIN FRACTIONS OF ORGANIC MATERIALS

Part of the ligneous fraction separated during the organic analysis of the saw-grass peat profile, sample No. 2, was used for testing its base capacity and was then subjected to repeated treatments with 30 per cent hydrogen peroxide (*20*). The base-capacity test was repeated on the residue which was later ashed. The results secured are given in Table 7.

TABLE 7.—*Base-exchange capacity of ligneous fractions of peat, a lignin from sample No. 2, Belle Glade peat colloids*

Depth of soil layer	Lignin fraction in colloid	Lignin ash in colloid	Base-exchange capacity of lignin			Dry residue after H_2O_2 treatment of colloid	Ashed residue after H_2O_2 treatment of colloid	Ash-free residue in colloid
			Per gram of colloid	Per gram of lignin	Residue, after H_2O_2 treatment per gram of colloid			
	Per cent	*Per cent*	*Milliequivalent*	*Milliequivalent*	*Milliequivalent*	*Per cent*	*Per cent*	*Per cent*
0–4 inches	44.25	0.771	0.080	0.181	0.016	3.29	2.46	2.83
4–6 inches	51.30	.906	.068	.128	.014	1.77	1.19	.58
32 inches	75.22	.722	.090	.120	.043	10.52	1.43	9.09
49 inches	74.91	.647	.115	.154	.045	10.43	4.01	6.42
63 inches	72.75	.832	.063	.108	.021	3.01	1.97	1.04
86–88 inches	59.92	2.962	.146	.241	.046	14.94	7.45	7.49

The base-exchange capacity of this ligneous material insoluble in various solvents appears to be larger than that of the residual inorganic soil colloids, yet it is less than was found in the organic soil colloid before fractionation. The extractions may destroy some organic oxide having base-exchange properties. Treatment with hydrogen peroxide removed most of the organic part of the ligneous fraction and destroyed most of its base-exchange capacity.

Similar tests with the ligneous fraction isolated from the willow-sedge peat colloid which had high base-exchange capacity further indicate that the presence of some inorganic material results in increased base-exchange capacity of ligneous material.

Tests of base-exchange capacity were made with ligneous material secured in the course of analyses of plant materials, as shown in Table 8. It appears from these tests that this isolated ligneous material does not necessarily possess as large a base-exchange capacity as the material from which it is derived.

TABLE 8.—*Base-exchange capacity of organic materials and of ligneous fractions separated from them*

Material	Base-exchange capacity per gram of plant material	Base-exchange capacity per gram of ligneous fraction	Ash in ligneous fraction
	Milli-equivalent	*Milli-equivalent*	*Per cent*
Sweetclover straw	0.125	0.141	3.26
Sphagnum moss, 0–2 inches	.490	.255	6.81
Sphagnum peat, 2–10 inches	.570	.459	7.51
Flax shives	.067	.131	1.69

BASE-EXCHANGE STUDIES WITH PLANT LIGNIN AND LIGNO-HEMICELLULOSE

Samples high in lignin and in hemicellulose were prepared from both sphagnum moss and flax shives by the method of Phillips (*15*). After the different extractions the hemicellulose was precipitated and washed with alcohol and the lignin boiled with hydrochloric acid to destroy any hemicellulose present, then washed free of chlorides. Samples of oat-hull lignin and corncob lignin were also used for base-exchange capacity determinations, with results as given below.

Material	Milliequivalent
Oat-hull lignin	0.062
Do	.050
Oat-hull lignin, second fraction	.039
Do	.038
Corncob lignin	.040
Do	.036
Flax-shive lignin:	
Wet	.192
Dry	.151
Flax-shive ligno-hemicellulose:	
Wet	.186
Dry [4]	.167
Sphagnum-moss lignin:	
Wet	.098
Dry	.041
Sphagnum-moss ligno-hemicellulose, wet [5]	.158

According to Phillips, lignin has a molecular weight of about 700 and apparently four hydroxyl groups to which a base might attach. Obviously, only a small fraction of the total theoretical base capacity at optimum reaction is manifested under the conditions of these tests.

[4] Contains 32.24 per cent hemicellulose.

[5] Contains 18.93 per cent hemicellulose.

From these determinations it appears that drying lowers the base-exchange activity of these substances. This may be due to diminution of ingress of soluble salts to the interior of particles. The tendency of these ligneous and lignocellulose fractions to include impurities and to hydrolyze under treatment of base-exchange tests is a cause of some difficulty and uncertainty.

GENERAL DISCUSSION

The data presented clearly indicate that a portion of the peat profile samples studied is of colloidal size. The particles retained in the centrifuge bowl show some colloidal properties, and it seems probable that the effective size for marked colloidality is larger for organic than for inorganic colloid. The tendency shown for additions of calcium carbonate to increase base adsorption suggests that such treatment may conserve or produce base-adsorptive complexes. Since the ligneous fraction in these experiments and those of Tenney and Waksman (*25, 26*) tends to become concentrated as decomposition of organic matter advances, and organic material, such as saw-grass peat or flax shives, of large ligneous content is slow to decay, it appears that the base-exchange capacity of organic residues is fairly permanent. The ligneous character and high base-exchange capacity of old sedimentary layers would appear to support this view.

Chopping or grinding, and control of temperature, moisture, aeration, reaction, and nutrients required by decomposition microorganisms, afford means of regulating the rate of decomposition (*28*). A knowledge of composition and base-exchange capacity of farm waste or available litter should be helpful. The flash decomposition of legume residues like sweetclover, noted by Smith and Humfeld (*23*), although they release nutrients and energy, may be wasteful under some circumstances.

When small amounts of peat or ligneous colloid are mixed with sand, the base-exchange activity of such organic colloids may increase with dilution until not more than a monomicellar layer of colloid coating surrounds each sand grain.

Mixing organic and inorganic colloid and supplying calcium carbonate may provide conditions favorable for a high base-exchange capacity of the whole system. A concentration of ions at the interface of a mixture may operate to increase base-exchange capacity. A large proportion of organic matter and the presence of bases sufficient to give a faintly alkaline reaction is indicated as desirable by these studies and is in line with results of Mattson (*13*). It seems possible that any acidic organic compound or other derivatives containing hydroxyl groups may react with bases, and that ketones or aldehydes may be oxidized to acids which would neutralize bases and perhaps allow base adsorption. Proteins or other amphoteric substances in soil organic matter may also conceivably affect base-exchange capacity of organic soil colloids. The organically combined and also the extraneous inorganic matter may affect the base-exchange capacity of the peaty colloids. Studies of natural or synthetic organic colloids seem a promising field for investigation. It would appear improbable that the base-exchange properties of natural organic soil colloid can be assigned to any definite chemical compound, since the material is the result of the reaction of widely different materials under very

diverse conditions. Comparative results under specified conditions are obtainable.

SUMMARY

Experiments with 8 peat profiles, including 26 soil-layer samples, show that it is possible to separate organic colloids from peat with fairly good, though scarcely quantitative, yields. This natural organic colloid absorbs about 50 per cent more water, over 3.3 per cent sulphuric acid, than the average for inorganic soil colloids. The proximate composition of peat colloids varies widely with parent material and climatic conditions. The colloid fraction is richer in nitrogen than the peat from which it is separated. Although the base-exchange capacity of peat is large, its colloid fraction gives much higher values. The ligneous fraction of the peat colloid, although manifesting comparatively good base-exchange capacity, appears to have this property largely destroyed by treatment with hydrogen peroxide.

Plant materials manifest base-exchange capacity in different degrees, which may affect their comparative values for green manure or stable litter. Studies with composts show a concentration of ligneous material as decomposition proceeds and this is correlated with increased base-exchange capacity. The state of the colloid, or of its ligneous fraction, appears to be conditioned for higher base adsorption by additions of sand, clay, or calcium carbonate. Formation of addition complexes, or the freeing of double-valence bonds, seems probable, and merits further investigation.

Results obtained should be of practical value in composting as well as in economy of soil organic matter and soil fertility.

LITERATURE CITED

(1) Alben, A. O.
1930. reactions of electrodialyzed humus and bentonite and application of the method. Jour. Amer. Soc. Agron. 22: 311–326, illus.

(2) Baver, L. D.
1930. the effect of organic matter upon several physical properties of soil. Jour. Amer. Soc. Agron. 22: 703–708.

(3) Demolon, A., and Barbier, G.
1929. conditions de formation et constitution du complexe argilo-humique des sols. Compt. Rend. Acad. Sci. [Paris] 188: 654–656.

(4) Doughty, J. L.
1930. the fixation of phosphate by a peat soil. Soil Sci. 29: 23–35, illus.

(5) Dunnewald, T. J.
1930. organic matter effects on soils. Jour. Amer. Soc. Agron. 22: 713–718.

(6) Feustel, I. C., and Byers, H. G.
1930. the physical and chemical characteristics of certain american peat profiles. U. S. Dept. Agr. Tech. Bul. 214, 27 p., illus.

(7) Gedroiz, K. K.
1918. [contributions to our knowledge of the absorption capacity of soils.] Zhur. Opytn. Agron. Iugo-Vostoka (Jour. Expt. Landw. Südost Eur.-Russlands) 19: 269–322. [Transl. by S. A. Waksman for C. Scofield, U. S. Dept. Agr.] [Original in Russian.]

(8) Halversen, W. V., and Torgerson, E. F.
1927. production of artificial farmyard manure by fermenting straw. Jour. Amer. Soc. Agron. 19: 577–584, illus.

(9) Hissink, D. J.
1926. The relation between the values pH, V and S (humus) of some humus soils, S (humus) and V of these soils with pH=7. The equivalent weight of the humus substance. Internatl. Soc. Soil Sci. Proc. 2nd Commn. A: [198]–207., illus.
(10) Humfeld, H.
1930. A method for measuring carbon dioxide evolution from soil. Soil Sci. 30: 1–10, illus.
(11) McGeorge, W. T.
1930. The base exchange properties of organic matter in soils. Ariz. Agr. Expt. Sta. Tech. Bul. 30, p. 182–213, illus.
(12) Marshall, C. E.
1927. Some recent researches on soil colloids. A review. Jour. Agr. Sci. [England] 17: [315]–332.
(13) Mattson, S.
1931. The laws of soil colloid behavior. V. Ion adsorption and exchange. Soil Sci. 31: 311–331.
(14) Odén, S.
1919. Die huminsauren. Kolloidchem, Beihefte 11: 75–260.
(15) Phillips, M.
1930. The chemistry of lignin. IV. Lignin from oat hulls. Jour. Amer. Chem. Soc. 52: 793–797.
(16) Powers, W. L.
1930. Chemical characteristics and utilization of some peat soils in northwestern United States. (Abstract) Northwest Sci. 4: 16–17.
(17) ———
1930. Chemical characteristics of peat and muck soils in northwestern United States. 2nd Internatl. Cong. Soil Sci. Proc. (in press)
(18) Robinson, W. O.
1930. Method and procedure of soil analysis used in the division of soil chemistry and physics. U. S. Dept. Agr. Circ. 139, 20 p.
(19) ———
1922. The absorption of water by soil colloids. Jour. Phys. Chem. 26: [647]–653.
(20) ———
1927. The determination of organic matter in soils by means of hydrogen peroxide. Jour. Agr. Research 34: 339–356.
(21) ——— and Holmes, R. S.
1924. The chemical composition of soil colloids. U. S. Dept. Agr. Bul. 1311, 42 p.
(22) Shorey, E. C.
1930. Some methods for detecting differences in soil organic matter. U. S. Dept. Agr. Tech. Bul. 211, 28 p.
(23) Smith, N. R., and Humfeld, H.
1930. Effect of rye and vetch green manures on the microflora, nitrates, and hydrogen-ion concentration of two acid and neutralized soils. Jour. Agr. Research 41: 97–123, illus.
(24) Smolik, L.
1930. The base exchange in soils rich in organic matter. Vestnik Ceskoslov. Akad. Zemědělské 6: 912–915.
(25) Tenney, F. G., and Waksman, S. A.
1929. Composition of natural organic materials and their decomposition in soil: IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials under aerobic conditions. Soil Sci. 28: 55–84, illus.
(26) ——— and Waksman, S. A.
1930. The composition of natural organic materials and their decomposition in the soil: V. Decomposition of various chemical constituents in plant materials under anaerobic conditions. Soil Sci. 30: 143–160, illus.

(27) TIDESWELL, F. V., and WHEELER, R. V.
1922. ON DOPPLERITE. STUDIES IN THE COMPOSITION OF COAL. Jour. Chem. Soc. Trans. 121 (pt. 2): 2345–2362, illus.
(28) WAKSMAN, S. A.
1929. CHEMICAL AND MICROBIOLOGICAL PRINCIPLES UNDERLYING THE DECOMPOSITION OF GREEN MANURES IN THE SOIL. Jour. Amer. Soc. Agron. 21: 1–18.
(29) ——— and STEVENS, K. R.
1929. CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: IV. CHEMICAL STUDIES OF HIGHMOOR PEAT PROFILES FROM MAINE. Soil Sci. 27: 389–398.
(30) WAY, J. T.
1850. ON THE POWER OF SOILS TO ABSORB MANURE. Jour. Roy. Agr. Soc. England 11: 313–379.

THE DECOMPOSITION OF VETCH GREEN MANURE IN RELATION TO THE SURROUNDING SOIL[1]

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INTRODUCTION

In two previous papers by Smith and Humfeld (*7*, *8*),[3] studies on the effect of the decomposition of green rye and green vetch, used as green manures on two acid and two neutralized soils, were reported. The results obtained indicated that further studies on the extent of the activities of the microorganisms that caused the decomposition of the organic materials added would be desirable.

In these experiments the green manure turned under was deposited in a layer approximately 5 inches below the surface of the soil. Sampling consisted in taking a number of cores of soil to a depth of 6 inches and then homogenizing the soil from these cores in order to obtain a representative sample. In this operation the green manure was thoroughly mixed with the soil, and if any difference in activity existed in the soil at various depths or in the green manure, these differences tended to be obscured or masked. It was suggested that if any information were to be gained as to the nature or possible localization of the activity of the microorganisms in the layer of green manure and in the soil at different depths, a special method of sampling would have to be developed.

Preliminary observations[4] indicated that there were great differences in the number of organisms in the soil and in the green manures. In order to obtain conclusive data on the relationships of the decomposing green manure to the surrounding soil the following experiment was planned.

SOIL AND MANURE

Two plots of Leonardtown clay loam were used, one neutralized with ground limestone and the other left in the naturally acid condition. These plots formed part of the series used for the previously reported experiments on green manures (*7*, *8*), but had had no treatment, being kept fallow and at optimum moisture with distilled water. The size of each plot was 1½ feet by 3 feet, and the soil was approximately 10 inches deep. The vetch used as green manure was grown on a separate bench in the greenhouse until approximately a maximum growth was obtained. This corresponds to the stage just before flowering. The vetch was cut close to the soil and 2,500 grams of

[1] Received for publication July 20, 1931; issued March, 1932.

[2] The method of sampling used in this work is a result of a suggestion by Charles Thom, principal mycologist, that a study of the decomposing green manure be made apart from the soil. The authors are indebted to him for this suggestion and for his interest and criticism of the work. They wish to express their appreciation to Daniel Ready, assistant scientific aid, for making the ammonia, nitrate nitrogen, and moisture determinations, and to George Irving, jr., under scientific helper, for making the pH determinations.

[3] Reference is made by number (italic) to Literature Cited, p. 120.

[4] Leaves and stems of rye and vetch were sieved out of subsamples of the green manure studied by Smith and Humfeld. These were examined microscopically by Charles Thom. The enormous number of bacteria found in and on these green materials made necessary a restudy of the whole situation on a different method of sampling; hence this paper.

the green vetch tops were brought to the experimental plots and turned under 5 inches below the surface of the soil. The vetch contained 13.8 per cent dry matter and 3.97 per cent total nitrogen on the dry basis. This is equivalent to about 53,500 pounds of green vetch per acre, or about 7,400 pounds of dry material and 270 pounds of nitrogen per acre.

The plots were sampled immediately after the vetch was turned under, subsequent samples being taken 2, 4, 7, 14, 21, 35, and 56 days after green manuring.

METHOD OF SAMPLING

The cores of soil were taken in the usual manner, but instead of mixing the sample obtained, each core was divided carefully into three equal parts. (1) The 0 to 2 inch layer, (2) the 2 to 4 inch layer, and (3) the 4 to 6 inch layer. This last layer contained the green manure, which was separated as thoroughly as possible from the soil and treated as a separate sample. In order to get material enough for analysis twenty 6-inch cores were taken from each plot and fractionated as described. The homologous fractions were combined, homogenized, and samples weighed out for the determination of the number of soil microorganisms and protozoa, amounts of ammonia nitrogen, nitrate nitrogen and moisture, the pH value of the soil and decomposing green manure, and the carbon dioxide evolution from the surface of the soil.

METHODS OF ANALYSIS

The number of microorganisms was estimated by plating the appropriate dilutions on soil-extract agar, as described previously (*7*). The number of protozoa was determined by the dilution method, using dilutions of 1 to 50, 1 to 500, 1 to 5,000, and, when necessary, 1 to 50,000. Tubes of broth [5] were inoculated with a milliliter of these dilutions and incubated 7 to 10 days at 28° C. The presence of protozoa was determined by microscopic examination. Five tubes were inoculated from each dilution. The number of protozoa in the original material was estimated from the number of tubes of the highest dilution showing a growth of protozoa. The counts are reported as number per gram of oven-dry material.

Ammonia nitrogen was determined by distillation with MgO according to the official methods (*1*), with the following modification: The NH_3 was collected in 5 per cent H_3BO_3 and was titrated with standard 0.14N H_2SO_4, brom phenol blue being used as an indicator, as described by Scales and Harrison (*6*) and Markley and Hann (*3*).

Nitric nitrogen in the soil samples was determined by the phenol disulphonic acid method. In the green manure it was determined by treating the residue from the ammonia analysis according to the Zn-Cu couple reduction method (*5*), boric acid being used as the absorbing agent and the titration being made as described for ammonia nitrogen.

The reaction of the samples was determined by means of the quinhydrone electrode in the usual manner. Moisture determinations were made by drying the samples at 105° C. overnight.

[5] Composition of the broth was as follows: Soil extract 1,000 cubic centimeters, and K_2HPO_4 0.5 gram. To each test tube containing about 8 milliliters of this broth, there was added a piece of timothy hay long enough to extend well above the surface of the liquid. Sterilization was by autoclaving.

Carbon dioxide evolution was determined as described by Humfeld (*2*). The method consists of passing a known volume of air over a known and inclosed area of soil and collecting it by means of absorption in potassium hydroxide solution of known strength. The amount of carbon, as carbon dioxide evolved from the soil, was calculated as grams per square meter in 24 hours.

RESULTS

The results of the plate counts of microorganisms in the soil and in the decomposing green manure are summarized in Table 1.

TABLE 1.—*Millions of microorganisms in soil at various depths when unlimed and limed and in fresh and in decomposing green manure*

[Calculated on the basis of dry weight]

Days after green manuring	Microorganisms in unlimed soil at indicated depth (inches)			Green-manure layer	Days after green manuring	Microorganisms in limed soil at indicated depth (inches)			Green-manure layer
	0 to 2	2 to 4	4 to 6			0 to 2	2 to 4	4 to 6	
	Millions	*Millions*	*Millions*	*Millions*		*Millions*	*Millions*	*Millions*	*Millions*
0	7.5	7.9	11.1	970	0	43.9	45.6	35.1	970
2	6.4	5.3	7.7	7,350	2	47.6	36.3	20.0	8,800
4	13.4	10.1	11.0	21,500	4	52.6	29.9	34.2	46,400
7	21.0	18.8	20.5	5,430	7	61.3	48.1	66.6	5,000
14	12.5	8.4	13.3	800	14	32.0	34.5	23.8	760
21	23.1	17.2	27.5	508	21	60.1	37.0	39.4	420
35	10.6	8.7	8.7	352	35	44.8	47.6	24.0	178
56	13.7	3.7	10.8	73.5	56	75.0	39.6	32.2	87.6

It will be noted that the number of microorganisms in the limed soil was considerably higher than the number in the unlimed soil, and that the fluctuations in number throughout the duration of the experiment were comparatively small. There was a tendency for the number in the different soil layers to increase, the highest counts being obtained in 7 and 21 days. However, when these counts are compared with the counts obtained in the green manure itself, they become insignificant, for while the number of microorganisms in the soil runs into the millions, the number in the green manure runs into the billions. The count of the vetch immediately after it was turned under was 970,000,000 per gram of dry material. This number is much greater than is ordinarily found on growing plants. It probably was due to the fact that the vetch had been grown in the greenhouse and had become closely matted on the soil. As a result, many of the lower leaves were dead. Under the optimum conditions for the growth of microorganisms in the greenhouse, these dead leaves were already undergoing decomposition and contained great numbers of bacteria and protozoa.

After four days the initial count had increased to more than 21,000,000,000 in the decomposing vetch in the acid soil and exceeded 46,000,000,000 in the limed soil. A rapid reduction in numbers took place after this, and 56 days after treatment only 73,000,000 and 88,000,000 were found in the acid and limed soil, respectively. The disappearance of the majority of the organisms in the green manure coincided with the disappearance of the leafy part of the vetch. It is apparent that when the more readily decomposable parts of the vetch had been consumed, the numbers rapidly decreased, as the more resistant stems did not contain sufficient readily available materials to support the great number of microorganisms present.

It is interesting to note the results obtained in counting the protozoa as shown in Table 2.

TABLE 2.—*Numbers of protozoa in soil at various depths when unlimed and limed and in fresh and decomposing green manure*

[Calculated on the basis of dry weight]

Days after green manuring	Protozoa in unlimed soil at indicated depth (inches)			Green-manure layer	Days after green manuring	Protozoa in limed soil at indicated depth (inches)			Green-manure layer
	0 to 2	2 to 4	4 to 6			0 to 2	2 to 4	4 to 6	
0	0	90	60	7,300	0	90	30	30	14,600
2	40	40	0	23,000	2	40	0	80	24,500
4	40	80	40	152,000	4	40	80	0	81,500
7	80	80	0	960	7	40	0	0	890
14	120	30	90	79	14	60	60	0	790
21	60	60	30	750	21	30	60	30	750
35	90	0	0	380	35	0	90	0	1,100
56	30	0	60	75	56	0	0	0	75

The number of protozoa in the different soil layers was small, the variation recorded being attributable to the method of counting. The vetch layer just after it was turned under contained a larger number, but the interesting fact was the increase in numbers of protozoa in the decomposing green manure. These numbers increased rapidly, the peak being reached in four days. After that the count dropped suddenly and remained low.

The number of fungi and actinomycetes was not determined, as all previous studies had shown that there is no significant change in the number of either under these conditions.

TABLE 3.—*Parts per million of ammonia nitrogen in unlimed and limed soil at various depths, and in fresh and in decomposing green manure*

[Calculated on the basis of dry weight]

Days after green manuring	Ammonia nitrogen in unlimed soil at indicated depth (inches)			Green-manure layer	Days after green manuring	Ammonia nitrogen in limed soil at indicated depth (inches)			Green-manure layer
	0 to 2	2 to 4	4 to 6			0 to 2	2 to 4	4 to 6	
0					0				
2			13	620	2	11	8	8	568
4	7	3	8	752	4	1	2	6	1,060
7	10	13	13	142	7	6	9	17	164
14	13	19	56	285	14	8	6	53	174
21	14	23	62	210	21	17	14	25	59
35	11	21	35	65	35	12	12	12	20
56	13	24	26	23	56	6			6

The results obtained from the analyses for ammonia nitrogen are given in Table 3 and show that the amount of this form of nitrogen in the soil was low. However, the amount in the green manure was considerable and was especially high in the first four days of decomposition. As decomposition progressed, the amount of ammonia nitrogen decreased, and at the end of the experiment the amount was no higher than in the surrounding soil. The ammonia nitrogen in the 4 to 6 inch layer, or in the soil adjacent to the green manure, increased to a maximum of 62 parts per million in 21 days in the unlimed soil and to 53 parts per million in 14 days in the limed soil. This was no doubt due to the diffusion of ammonia from the decomposing material.

TABLE 4.—*Parts per million of nitric nitrogen in unlimed and limed soil at various depths, and in fresh and in decomposing green manure*

[Calculated on the basis of dry weight]

Days after green manuring	Nitric nitrogen in unlimed soil at indicated depth (inches)			Green-manure layer	Days after green manuring	Nitric nitrogen in limed soil at indicated depth (inches)			Green-manure layer
	0 to 2	2 to 4	4 to 6			0 to 2	2 to 4	4 to 6	
0	33	39	34	----------	0	59	65	49	----------
2	42	37	35	608	2	35	70	31	570
4	43	39	40	429	4	70	53	41	477
7	62	35	38	30	7	109	51	46	25
14	88	36	36	44	14	134	49	95	59
21	82	42	51	50	21	139	78	88	36
35	116	47	54	74	35	182	67	83	86
56	198	64	60	67	56	439	93	71	74

The nitric nitrogen, as shown in Table 4, in the unlimed soil at the beginning of the experiment was 33 to 39 parts per million; the limed soil contained 49 to 65 parts per million.

In the green manure the nitric nitrogen decreased rapidly, and after seven days only 30 and 25 parts per million were present in the green-manure layer of the acid and limed soils. The outstanding fact is the gradual accumulation of nitric nitrogen of the upper 2-inch layer. In the unlimed soil the increase was from 33 to 198 parts per million, and in the limed soil, from 59 to 439 parts per million.

TABLE 5.—*pH values of the unlimed and limed soil at various depths and of the fresh and decomposing green manures*

Days after green manuring	pH value of unlimed soil at indicated depth (inches)			Green-manure layer	Days after green manuring	pH value of limed soil at indicated depth (inches)			Green-manure layer
	0 to 2	2 to 4	4 to 6			0 to 2	2 to 4	4 to 6	
0	4.3	4.3	4.3	6.8	0	7.3	7.4	7.0	6.7
2	4.2	4.3	4.3	5.5	2	7.3	7.5	6.8	7.3
4	4.2	4.3	4.2	7.3	4	7.5	7.6	6.9	8.1
7	4.1	4.3	4.3	4.7	7	7.2	7.2	6.5	7.6
14	4.2	4.4	4.8	6.6	14	7.2	7.4	7.1	7.7
21	4.1	4.2	4.4	5.5	21	7.2	7.4	6.7	6.6
35	4.0	4.3	4.3	4.6	35	7.3	7.6	7.3	7.3
56	3.9	4.1	4.2	4.3	56	7.0	7.4	7.2	6.8

Table 5 shows the results of the pH determinations. The pH value of the green manure added to the unlimed soil was 6.8, whereas the pH value of the soil was 4.3. However, as the vetch disappeared, the pH value of the green manure decreased, and at the last sampling it was the same as that of the surrounding soil. The surface layer in the meantime had become more acid, perhaps due to the concentration of nitrates there.

In the limed soil the pH value of the green manure was somewhat higher than that of the surrounding soil during the stage of most active decomposition and during the greatest accumulation of ammonia. The difference was not so striking as in the case of the unlimed soil.

The rate of carbon dioxide production is given in Figure 1. The effect of turning under green manure on the evolution of carbon

dioxide was immediate and was marked. The peak of the evolution was reached in 3 days, after which a rapid reduction took place. In 20 days this decline had practically stopped, and a fairly constant rate, which was close to the rate prevailing before the green manure was turned under, was maintained. Apparently the quantity of material turned under was the determining factor. The amount of carbon dioxide given off from both limed and unlimed soil before treatment was negligible when compared to that given off after the addition of the green manure.

DISCUSSION

In order to gain a clear picture of the effect of the addition of green vetch to this soil, it is necessary to compare the results of each determination with those of every other determination. For instance, if plate counts of microorganisms are compared with the other

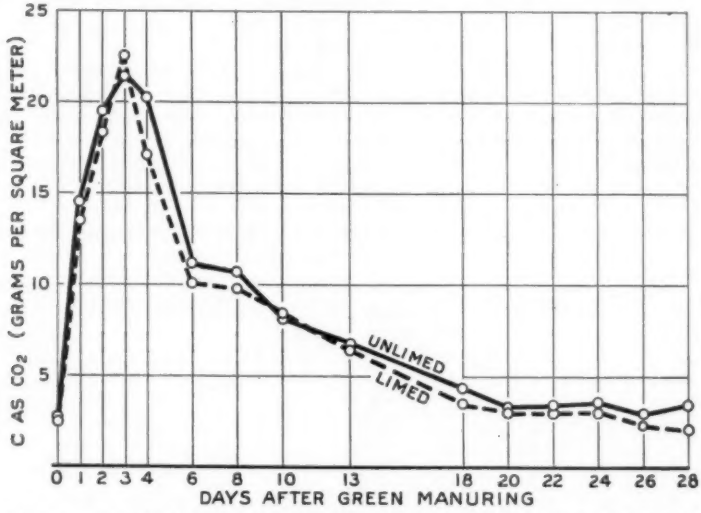

FIGURE 1.—Grams C as CO_2 evolved per square meter of limed and unlimed soil treated with vetch green manure

factors certain correlations are seen. When the plate counts of microorganisms in the green-manure layer were high, as during the first few days, the count of protozoa, the amount of ammonia nitrogen, and the evolution of carbon dioxide were also high, as might be expected. Consequently, at the same time the pH value of the green manure was at the maximum and the nitric-nitrogen content, although still high, was being rapidly reduced.

The decomposition of the green manure causing the great increase in number of microorganisms, which by observation of the plates were found to be almost exclusively bacteria, had practically no effect on the number of microorganisms in the soil itself. Since the green manure contained a great number of microorganisms before it was turned under, it may be said that its decomposition proceeded

independently of the microorganisms in the soil. The same observation may be made as to the number of protozoa. Whether the plant material itself served as a source of energy for the protozoa or whether, as has been suggested by Russell (*4*), the protozoa subsist on bacteria, can not be stated. However, it was definitely determined that as the number of bacteria increased the number of protozoa increased and also as the number of bacteria decreased the number of protozoa decreased.

The increase in ammonia-nitrogen content of the green manure was no doubt due to the breaking down of the more complex nitrogen compounds in the plant material. Isolation of a number of the bacteria from the colonies obtained from the plate counts of this material showed that most of them were able to ammonify peptone broth readily. This ammonification of the proteins in the plant materials caused an increase in the pH of the material and was, of course, at a maximum when microbial activity was greatest.

The nitric-nitrogen determinations, however, give a somewhat different picture. In the green manure the nitric nitrogen was highest at the beginning and decreased rapidly up to seven days after the vetch was turned under. After that it remained approximately constant. It is evident that nitrification of the ammonia was constantly taking place, because, although the amount of ammonia in the soil adjacent to the green manure was always somewhat higher after the first seven days than in the soil more distant from the green-manure layer, an accumulation of ammonia did not take place, but a gradual accumulation of nitric nitrogen occurred in the upper 2 inches of soil. This accumulation took place both in the naturally acid and in the limed soil. It is very evident that although the soil of the unlimed plot reached a very low pH value (3.9), nitrification was sufficiently rapid to insure the oxidation of the ammonia given off by the green manure.

It is not clear from the data at hand whether the increase in nitric nitrogen was due to nitrification at or near the surface of the soil, or whether the accumulation was the result of nitrification at greater depths and the transportation of the nitrates to the surface by the physical action of capillarity and surface evaporation. Some observations as to the concentration of nitrates at the surface under the conditions obtaining during this and similar experiments have shown that a great proportion of the total nitrates in the soil may be concentrated at the surface. Concentrations of 1,300 parts per million have been found in the upper one-quarter inch of soil. It has been assumed that the nitrates were carried to the surface by capillary action, where they were left by the evaporation of the water. Periodic sprinkling should dissolve these nitrates and distribute them through the soil, but apparently it did not do so.

As the number of microorganisms in the green manure decreased, the nitric-nitrogen accumulation in the upper 2 inches of soil increased. This was in agreement with previous findings (*7*, *8*).

The carbon-dioxide evolution correlated very nicely with the other activities, the peak of carbon-dioxide evolution coinciding with the time at which the greatest number of microorganisms were found in the green manure. It may be said that under the conditions of the experiment the carbon-dioxide evolution was a good indication of the microbial activity in the material undergoing decomposition.

SUMMARY

An experiment on the decomposition of green vetch added to a naturally acid and a limed soil is reported.

A special method of sampling was used in which the 6-inch cores of soil were divided in three successive 2-inch layers. The third layer contained the layer of green manure, which was separated from the soil as thoroughly as possible and treated as a separate sample.

Twenty fractionated cores from each plot were homogenized, and the representative samples of each layer obtained were analyzed for number of microorganisms as indicated by plate count on soil-extract agar, number of protozoa, and amount of ammonia nitrogen, nitric nitrogen, and moisture. The soil reaction (pH) and carbon-dioxide evolution were also determined.

The results show that the increase in number of bacteria and protozoa was limited almost entirely to the green-manure layer. The soil acted as a blanket, insuring more or less uniform conditions of temperature and moisture, and as an absorbent for the ammonia produced in the decomposition of the green manure. In all probability the soil was the material in which nitrification of this ammonia took place. It was also found that rapid nitrification took place even in the very acid soil and that an accumulation of nitrates occurred at the surface.

The pH of the green manure was shown to be higher than the pH of the surrounding soil during the period of active decomposition. Carbon-dioxide production was correlated with microbial activity and was considered to be a good indicator of such activity under the conditions of the experiment.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis compiled by the committee on editing methods of analysis, revised to july 1, 1924. Ed. 2, 525 p., illus. Washington, D. C.

(2) Humfeld, H.
1930. a method for measuring carbon dioxide evolution from soil. Soil Sci. 30: 1–10, illus.

(3) Markley, K. S., and Hann, R. M.
1925. a comparative study of the gunning-arnold winkler boric-acid modifications of the kjeldahl method for the determination of nitrogen. Jour. Assoc. Off. Agr. Chem. 8: 455–467, illus.

(4) Russell, Sir E. J., [and others.].
1923. the micro-organisms of the soil. 188 p., illus. London, New York [etc.].

(5) Scales, F. M.
1916. a method for the determination of nitric nitrogen. Jour. Biol. Chem. 27: 327–337, illus.

(6) ——— and Harrison, A. P.
1920. boric acid modification of the kjeldahl method for crop and soil analysis. Jour. Indus. and Engin. Chem. 12: 350–352.

(7) Smith, N. R., and Humfeld, H.
1930. effect of rye and vetch green manures on the microflora, nitrates, and hydrogen-ion concentration of two acid and neutralized soils. Jour. Agr. Research 41: 97–123, illus.

(8) ——— and Humfeld, H.
1931. the decomposition of green manures grown on a soil and turned under compared to the decomposition of green manures added to a fallow soil. Jour. Agr. Research 43: 715–731, illus.

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN CROSSES OF WHITE FEDERATION WITH TURKEY WHEATS [1]

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INTRODUCTION

In the study of the inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., it is desirable to know the number of resistant factors present in each resistant variety and the effect of each factor. Also it is necessary to know whether or not various resistant varieties contain the same or different factors for resistance if such varieties are to be used intelligently for breeding other resistant varieties. Crosses are made with appropriate test varieties, as soon as they are available, in order to determine this last point. Martin wheat (*1*)[3] has a single dominant factor for resistance to bunt and is used as a test variety for this factor, which is designated as the Martin factor. The only other test variety now available is selection 1418. This selection contains the second Hussar factor (*3*) which allows bunt to develop on about half the heterozygous plants. However, this selection was not available when the investigations with Turkey C. I.[4] 1558 and Turkey C. I. 3055 were begun.

This paper deals with crosses of susceptible White Federation with resistant Turkey C. I. 1558 and Turkey C. I. 3055 wheats from which the number of factors for resistance and their effect may be determined. These two Turkey wheats also were crossed with Martin to see whether the Martin factor was present. Recently appropriate crosses were made to determine whether or not the factors present in these two Turkey wheats were identical with each other and the same as the second Hussar factor. These data will not be available for two or three years.

The literature relating to inheritance of resistance to bunt in wheat has been reviewed and discussed in previous publications (*1*, *3*, *4*).

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there favor such investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winter killing and with the assurance that both types will mature the following summer.

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[2] The writer acknowledges valuable suggestions from R. E. Clausen, Division of Genetics, and various members of the Division of Agronomy, University of California, and from various members of the Division of Cereal Crops and Diseases.

[3] Reference is made by number (italic) to Literature Cited, p. 126.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The seeds were thoroughly blackened with bunt spores. The inoculum was collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hills district of Solano County, Calif. It was propagated by Mackie on Little Club wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has grown bunt from this original collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. Since Faris (*5*) has shown that physiologic forms of bunt exist, the writer has been careful not to introduce new collections of bunt into the nursery. The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same physiologic form, or possibly a mixture of forms, has been employed in all these bunt investigations. This is indicated also by the fairly constant way in which the parental wheat varieties have reacted to this inoculum. This collection of bunt has been designated by Reed (*6*) as Physiologic Race III of *Tilletia tritici*.

The wheat seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown within three or four days in order to avoid the effects of different temperatures and soil moistures. At harvest time the plants in each row were pulled and separated into two piles, bunt free and bunted. The total number of plants and the number of bunted plants were recorded, and the percentage of bunt infection was calculated. A plant was classified as bunted if it showed any infection.

The percentages of bunt produced by the parent varieties may be seen in Table 1.

TABLE 1.—*Annual percentage of bunt infection in the parent wheat varieties from 1920 to 1922 and 1927 to 1929, when grown at Davis, Calif.*

Variety	Percentage of bunted plants						
	1920	1921	1922	1927	1928	1929	Average
Turkey C. I. 1558	14.5	0.3	0.5	0	2.8	0	3.00
Turkey C. I. 3055	0	0	0	0	.2	.1	.05
Martin	0	0	0	0	0	0	0
White Federation	88.7	51.6	58.3	66.6	68.9	78.6	68.80

Turkey C. I. 1558 had 14.5 per cent of bunted plants in 1920. Since that time the percentage of bunt in this variety has been much lower, reaching a maximum of 2.8 per cent in 1928. The reasons for the comparatively high percentage of diseased plants in 1920 are not apparent. Tisdale et al. (*7*) report an average of almost 9 per cent of bunted heads for this variety at Moro, Oreg., in 1919 and 1920.

Turkey C. I. 3055 has been almost free from bunt, producing only a little in 1928 and 1929. This variety produced 1 per cent of bunt in 1919 but was bunt free in 1920 at Moro, Oreg. (*7*). Martin has been entirely free from bunt at Davis, while White Federation, the susceptible parent, has produced more than 50 per cent of diseased plants each year.

EXPERIMENTAL RESULTS

All the crosses were made in 1926. The F_1 seeds were not inoculated because of the small number available.

A part of the F_2 seeds of all crosses was treated with copper carbonate to protect them from bunt infection so that a supply might

be grown for F_3. Enough seeds to plant approximately 20 rod rows of each cross were inoculated and grown in 1928.

The F_2 data do not permit a satisfactory genetic analysis because some susceptible plants usually escape infection. Some resistant plants occasionally also become partly infected. The data do give some idea of the number of factors present and indicate the percentage of bunted plants that may be expected in F_3 rows of the same genotype. The data collected in F_2 are recorded in Table 2.

TABLE 2.—*Percentage of bunted plants in parents and F_2 of the crosses named when grown in the field at University Farm, Davis, Calif., 1929*

Parent or cross	Total plants	Bunted plants	
	Number	*Number*	*Per cent*
Turkey C. I 1558	504	14	2.8
Turkey C. I. 3055	479	1	.2
White Federation	309	253	81.9
White Federation × Turkey C. I. 1558	815	421	51.6
White Federation × Turkey C. I. 3055	623	252	40.4
Martin × Turkey C. I. 1558	921	43	4.7
Martin × Turkey C. I. 3055	1,016	50	4.9

There was 51.6 per cent of bunted plants in the F_2 of White Federation × Turkey C. I. 1558 as compared with 40.4 per cent in the cross with Turkey C. I. 3055. The selection 1418, which carries the second Hussar factor, produced 53.3 per cent of bunted plants in F_2 when crossed with Little Club (*3*). The F_2 data, then, indicate that both strains of Turkey contain single factors for resistance to bunt which are similar in effect to the second Hussar factor.

The F_2 of Martin × Turkey C. I. 1558 and Martin × Turkey C. I. 3055 contained 4.7 and 4.9 per cent of bunted plants, respectively, showing that the Martin factor is not present in these varieties.

In the F_3, 299 rod rows were grown from 299 F_2 plants of the cross White Federation × Turkey C. I. 1558. There were grown also 296 F_3 rows of White Federation × Turkey C. I. 3055, 183 rows of Martin × Turkey C. I. 1558, and 190 rows of Martin × Turkey C. I. 3055. The F_2 plants from which these F_3 rows were grown had been protected from bunt by seed treatment. There were from 30 to 60 plants in each F_3 row. The classification of F_2 plants on the basis of the behavior of their progeny in F_3 rows is more reliable than classification in F_2. The F_3 data are shown in Table 3.

The number of rows in the 0 to 5 per cent class were separated into those with no bunted plants and those with 1 to 5 per cent of bunted plants because of the special interest in the former. The nature of the distribution of White Federation × Turkey may be seen more readily from Figure 1. In each cross the number of rows under the three modes agrees satisfactorily with the 1:2:1 ratio. Accepting the minima as they occur, the number of rows for White Federation × Turkey C. I. 1558 was 79.0:154.5:76.5 where the number expected was 77.5:155:77.5. In the cross with Turkey C. I. 3055 there were 64.5:155.0:76.5 where 74:148:74 were expected. The minima perhaps should not be thought of as representing with absolute accuracy the divisions between phenotypes. The agreement between the two crosses is good, and the minima fall practically at

the same points as those obtained in the cross with the second Hussar factor (*3*). In the latter case the first minimum occurred at 17.5 and the second one at 67.5 per cent of bunt infection.

TABLE 3.—*Distribution of parent and F_3 rows of the crosses named into 5 per cent classes for bunt infection, when grown at Davis, Calif., 1929*

Parent or cross	Distribution of rows by percentage classes for bunt infection										
	0	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50
Turkey C. I. 1558	12										
Turkey C. I. 3055	11	1									
White Federation											
Martin	6										
White Federation × Turkey C. I. 1558	27	34	10	6	4	6	9	24	31	27	21
White Federation × Turkey C. I. 3055	36	19	8	3	6	17	25	28	24	19	15
Martin × Turkey C. I. 1558	73	24	29	16	12	11	6	1	3	2	2
Martin × Turkey C. I. 3055	89	38	17	16	12	4	3		2		2

Parent or cross	Distribution of rows by percentage classes for bunt infection										Total number rows
	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100	
Turkey C. I. 1558											12
Turkey C. I. 3055											12
White Federation	1	1				1	8	7	4	1	23
Martin											6
White Federation × Turkey C. I. 1558	17	10	6	3	6	8	9	23	13	5	299
White Federation × Turkey C. I. 3055	10	6	2	3	2	6	17	26	16	8	296
Martin × Turkey C. I. 1558		1	1		2						183
Martin × Turkey C. I. 3055		1	1		2		1		1	1	190

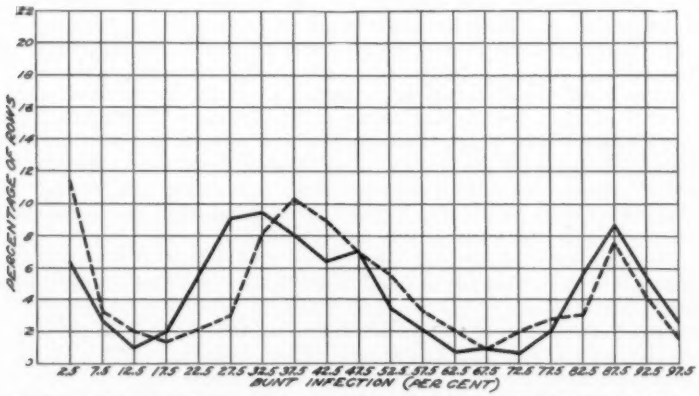

FIGURE 1.—Distribution of F_3 plants on the basis of F_3 rows of the crosses White Federation × Turkey C. I. 1558 wheat (broken line) and White Federation × Turkey C. I. 3055 wheat (solid line) into 5 per cent classes for bunt infection

Although the agreement between the two crosses is good, it is obvious that there is a higher percentage of bunt in the heterozygous F_3 rows of the Turkey C. I. 1558 cross than in the Turkey C. I. 3055 cross. The former had an average of 41.5 per cent of bunted plants and the latter 36.3. It will be recalled that the F_2 of White Federa-

tion × Turkey C. I. 1558 produced 51.6 per cent of diseased plants, as compared with 40.4 in the Turkey C. I. 3055 cross. The reason for the more frequent occurrence of bunt on heterozygous plants is not known definitely but it may be due in part to modifying factors. In Table 1 Turkey C. I. 1558 was shown to be a little more susceptible to bunt than Turkey C. I. 3055. That small differences in the amount of bunt in resistant wheats may be due to the presence of modifying factors has been shown in an earlier publication (*2*). If the higher percentage of bunt in heterozygous rows of White Federation × Turkey C. I. 1558 is due to the presence of modifying factors, the percentage of bunt in resistant and susceptible rows likewise should be higher. The resistant rows of the Turkey C. I. 1558 cross have an average of 3.1 per cent of bunt as compared with 1.9 per cent for the Turkey C. I. 3055 cross, but the reverse is true for the susceptible rows. The former cross had 84 per cent of diseased plants as against 86.9 per cent for the Turkey C. I. 3055 cross. The differences are not great and do not change the general conclusions that Turkey C. I. 1558 and Turkey C. I. 3055 differ from White Federation in one main factor for resistance. These factors are similar to each other in effect and also are similar in effect to the second Hussar factor. Investigations are under way to determine whether these factors are identical with each other and are the same as the second Hussar factor.

The data obtained from the crosses of White Federation with Turkey C. I. 1558 and Turkey C. I. 3055 demonstrate clearly that the factors for resistance to bunt in these two strains of Turkey are different from the factor in Martin. Because the crosses with Martin were available, they were carried through the F_3. The presence of bunt in F_2 of Martin × Turkey C. I. 1558 and Martin × Turkey C. I. 3055 and the presence of susceptible and segregating rows in F_3 of these crosses (Table 3) confirm the conclusion that the Martin factor is not present in either of these strains of Turkey wheat.

SUMMARY

The bunt inoculum used in these experiments was derived from one original collection, designated by Reed as Physiologic Race III of *Tilletia tritici*.

Turkey C. I. 1558 and Turkey C. I. 3055, the resistant parents, are very resistant to bunt as compared with White Federation wheat, which produced more than 50 per cent of diseased plants under the conditions of these experiments.

Turkey C. I. 1558 and Turkey C. I. 3055 were crossed with White Federation to determine the number of factors for resistance to bunt in these strains of Turkey and to find out the effect of these factors. They were crossed with Martin to see whether they contained the Martin factor for resistance to bunt.

In the crosses with White Federation the classification of F_2 plants on the basis of the behavior of their progeny in F_3 rows showed that Turkey C. I. 1558 and Turkey C. I. 3055 each differ from White Federation in one main factor for resistance to bunt. These factors are similar in effect to each other and resemble the second Hussar factor in that about half the heterozygous plants become infected. Therefore, they differ from the factor for resistance to bunt in Mar-

tin, which is completely dominant. This point is confirmed further by the presence of bunt in F_2 of crosses of Martin with Turkey C. I. 1558 and Turkey C. I. 3055 and by the presence of susceptible and segregating F_3 rows of the same crosses.

Investigations are under way to determine whether or not the factor in Turkey C. I. 1558 is identical with the factor in Turkey C. I. 3055 and the same as the second Hussar factor.

LITERATURE CITED

(1) Briggs, F. N.
1926. Inheritance of resistance to bunt, Tilletia tritici (Bjerk.) Winter, in wheat. Jour. Agr. Research 32: 973–990, illus.

(2) ———
1929. Factors which modify the resistance of wheat to bunt, Tilletia tritici. Hilgardia 4: [175]–184, illus.

(3) ———
1930. Inheritance of the second factor for resistance to bunt, Tilletia tritici, in Hussar wheat. Jour. Agr. Research 40: 225–232, illus.

(4) ———
1930. Inheritance of resistance to bunt, Tilletia tritici, in White Odessa wheat. Jour. Agr. Research 40: 353–359, illus.

(5) Faris, J. A.
1924. Factors influencing the infection of wheat by Tilletia tritici and Tilletia laevis. Mycologia 16: 259–282, illus.

(6) Reed, G. M.
1928. Physiologic races of bunt of wheat. Amer. Jour. Bot. 15: 157–170.

(7) Tisdale, W. H., Martin, J. H., Briggs, F. N., Mackie, W. W., Woolman, H. M., Stephens, D. E., Gaines, E. F., and Stevenson, F. J.
1925. Relative resistance of wheats to bunt in the Pacific Coast States. U. S. Dept. Agr. Bul. 1299, 30 p.

FLOWERING BEHAVIOR OF THE HOG PEANUT IN RESPONSE TO LENGTH OF DAY [1]

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INTRODUCTION

One of the most interesting wild plants of the flora of the District of Columbia is the legume known as the hog peanut, *Falcata comosa* (L.) Kuntze. This plant grows best in rich damp soil in woods and thickets. In the writer's garden, developed by reclaiming a magnolia bog, this plant has stubbornly persisted as a most troublesome weed.

In Gray's Manual of Botany [2] it is stated that the hog peanut is a low and slender perennial. This plant as observed by the writer at Washington, D. C., is invariably an annual. This observation agrees with that of Adeline F. Schively,[3] who made a very careful study of its flowering behavior many years ago in Pennsylvania.

KINDS OF FLOWERS NORMALLY PRODUCED IN THE WILD STATE IN SUMMER

In many respects the flowering behavior of the hog peanut is unique. The production of flowers and seed is especially striking, since it is usual for the plants to produce each season flat aerial pods with small dry seeds, and in addition indehiscent subterranean pods with large fleshy seeds of distinctive character.

In the course of the season the hog peanut normally produces several types of blossoms, ranging on the one hand from strictly open, showy blue or whitish chasmogamic blossoms in small aerial racemes to strictly cleistogamic blossoms, some of which, on the tips of long slender stems, bury themselves in the upper layers of the soil and produce the true hypogean beans of the large fleshy type.

Among the more distinct forms of floral expression in the blossoming series several may be mentioned. The showy colored chasmogamic blossoms of midsummer, which develop as aerial flowers from the uppermost branches of the plant, appear to be the fully developed blossoms of the species. From the higher branches of the plant aerial greenish cleistogamic blossoms are likewise produced. Both types of aerial blossoms produce small, dry, dark-colored aerial pods with one to three small hard seeds. The showy perfect flowers appear only during the midsummer season. Plants grown in the greenhouse in winter never produce the blue showy type of flower, but greenish cleistogamic flowers develop and form small thin orbicular pods.

[1] Received for publication July 14, 1931; issued March, 1932.

[2] [GRAY, A.] GRAY'S NEW MANUAL OF BOTANY. A HANDBOOK OF THE FLOWERING PLANTS AND FERNS OF THE CENTRAL AND NORTHEASTERN UNITED STATES AND ADJACENT CANADA. Rearranged and ext. rev. by B. L. Robinson and M. L. Fernald. Ed. 7, 926 p., illus. New York, Cincinnati [etc.]. 1908. (See p. 530.)

[3] SCHIVELY, A. F. CONTRIBUTIONS TO THE LIFE HISTORY OF AMPHICARPAEA MONOICA. Penn. Univ. Contrib. Bot. Lab., 1: 270-363, illus. 1897.

——— RECENT OBSERVATIONS ON AMPHICARPAEA MONOICA. Penn. Univ. Contrib. Bot. Lab., 2: 20-30. 1904.

These are even smaller, more orbicular, and less obviously stalked than the pods produced by the similar greenish cleistogamic summer flowers.

In addition to the aerial cleistogamic flowers of summer and winter, long filiform stems bearing at their tips extremely reduced cleistogamic blossoms are formed. These normally push beneath the soil and produce the large fleshy subterranean beans. These beans are produced very abundantly under specific conditions during the entire summer and likewise constitute the common form of floral expression on winter-grown plants.

It would appear that the entire series of flowers merely represents more or less distinct gradations of floral reduction from the normal showy colored blossoms to the merest rudiments found in the most extremely reduced hypogean forms. So complete can this floral reduction become that even the number and size of the stamens and the stigma itself are affected. In the strictly subterranean flowers there is little indication of the normal elongate style, and the capitate stigma seen in the blue flowers is entirely wanting.

The fact that several types of flowers and beans are normally produced in a rather definite sequence throughout the season led Schively to investigate the behavior of the plant for the purpose of determining the factors responsible for these reproductive differentiations. Her observations and work make a very interesting contribution to the unique behavior of this wild bean; but since at the time of her investigations nothing was known of the length-of-day responses of plants, she failed to recognize any specific response that could be attributed to definite quantitative conditions of the seasonal environment.

SEASONAL RELATIONSHIPS OF FLOWERING

Consideration may now be given the seasonal relationships of flowering and the normal sequences observed for the production of the several types of flowers from the time the beans first germinate in late April until the plants die in autumn.

In a locality where the hog peanut grows abundantly a heavy crop of viable hypogean seed is usually produced. These are actually self-sown, as the blossom-tipped axillary runners bury themselves wherever possible in the uppermost soil layers and these yield a crop of hypogean beans. Plants may arise from the smaller aerial seed, but the viability and germination of these seeds from aerial blossoms seem far less positive.

Toward the last of April certain areas of the writer's garden are covered with thick stands of young hog-peanut plants arising from the abundant hypogean beans buried in the soil. The cotyledons of the hog peanut, unlike those of the soybean or the common garden beans, remain buried beneath the soil. As the young plants grow in May and June, a tangle of competing vines is produced, and the struggling plants become more or less vigorous climbers when they find at hand supporting weeds and shrubbery.

In mid June or early July, slender, drooping filiform runners sometimes appear, arising even from the shoots that develop from the cotyledonary axils beneath the soil. From the time these first runners with their extremely rudimentary flowers are developed runners are produced in abundance from the aerial leaf axils until

the end of the season, or as long as the plants remain in a vigorous growing condition. There is considerable individual variation, however, in the time of first appearance of these filiform stems with their cleistogamic flowers. On some plants their appearance is delayed as late as the last week of July or early August. This behavior is not anomalous, however, for the normal field assemblage, not having undergone rigorous natural selection for particular degrees of earliness or lateness, would be likely to include individuals of rather wide variability in this respect.

Several weeks after the filiform runners with the hypogean type of blossoms appear, aerial flowers, either greenish and cleistogamic or perfect and bluish colored, usually make their appearance. Schively [4] states that the aerial cleistogamic flowers do not appear until the showy blue aerial flowers are in evidence. This is not an invariable sequence, however, for the writer has observed that the greenish, more reduced cleistogamic aerial flowers have occasionally preceded a little the showy aerial ones, sometimes making their appearance during the first week of August, while the blue flowers did not appear until about August 20. Near the middle of August the blue aerial flowers appear either singly or in racemes.

WINTER FLOWERING IN THE GREENHOUSE

Naturally the winter type of aerial cleistogamic blossoms must be confined to artificially grown greenhouse material. However, it would appear that this type of flower is in some respects only a more reduced and more rudimentary floral structure than that produced by the plants in summer, when growth is favored by conditions of better illumination, long days, and perhaps more favorable moisture and temperature conditions. There is reason to believe that the winter type of cleistogamic flower and the resulting legume are but a near approach to the more extreme hypogean type produced upon the filiform runners. This is evidenced by the more extreme reduction in certain features of the style and stigma, and in the smaller, more orbicular pods, that, like the hypogean pods, usually contain but one seed.

RESPONSE OF THE PLANTS TO LENGTH OF DAY IN SUMMER

In 1925 and again in 1930 studies were made of the response of the hog peanut to different lengths of day. These studies have afforded a clearer understanding of the normal field behavior of the plant in a wild state. They have also suggested reasons for some of the floral differentiations and apparently established floral sequences throughout the season.

Tiny plants which had germinated from hypogean beans in late April and early May were taken from the wild state and planted in buckets. These were subjected to the following series of day lengths: 5, 8, 10, 12, 13, and 13½ hours and the full length of day of summer at Washington, D. C., i. e., 14 hours and 54 minutes from sunrise to sunset. In one test the plants were darkened from 10 a. m. to 2 p. m. each day.

Filiform runners bearing extremely rudimentary flowers were soon produced on plants under all lengths of day except the controls (full

[4] SCHIVELY, ADELINE F. Op. cit., p. 325. (See footnote 3.)

day) and the plants darkened in the middle of the day. In experiments carried out in 1930 with the different day-length treatments beginning May 2, filiform stems appearing May 29 reached the following lengths on June 2:

	Inches
8 hours daylight	5
10 hours daylight	4
12 hours daylight	3½
13 hours daylight	5½
13½ hours daylight	1½

Closed aerial flowers appeared on plants growing under the shortened day lengths on the following dates: On the 8-hour, 10-hour, and 12-hour days, June 10; 13-hour day, June 12; 13½-hour day, June 14. (Fig. 1.)

Showy, bluish, completely developed flowers appeared only on the plants experiencing a day length of 13½ hours and the full length of day. On the plants exposed to a 13½-hour day the flowers appeared on June 23 but on those exposed to full length of day the flowers did not appear until August 12. At no time during the season did the plants darkened in the middle of the day produce the showy bluish type of flower.

Filiform stems began to develop on these plants and on the control plants simultaneously on July 22, followed somewhat later by the closed aerial greenish cleistogamic flowers.

Figure 2 affords a comparison of plants grown under different lengths of exposure to daylight.

These tests and earlier experiments conducted in 1925 indicate that the length of day exerts an immediate and profound influence upon the type of blossoms produced by the hog peanut.

It is evident that an appropriate length of day not only controls the type of floral expression but also completely changes the normal sequences of flowering behavior as usually observed in wild plants in the field. Lengths of day of 13½ hours or less have favored a rapid development of axillary filiform stems bearing the extremely reduced hypogean type of blossoms. A length of day of not less than 13½ hours was required to produce the perfect showy aerial type of blossoms. The green cleistogamic summer type of blossom appears to represent an intermediate form not far removed from the showy flowers, since they are normally produced on the control plants very near the time when the showy blossoms themselves appear. These showy flowers, representing the typical fully developed blossoms of the hog peanut, are obviously favored by the longer midsummer day obtaining in this region.

The extremely rapid growth and excessive production of filiform stems bearing the hypogean type of flower is a remarkable response of the plants to the shorter lengths of day. Day lengths of 13 and 13½ hours were especially favorable to the growth of these runners or stolonlike stems, as shown in Figures 1 and 2. This excessive runner growth was coincident with a more rapid and vigorous growth of the plants themselves in leafiness and in stature. It would appear that the greatest reduction toward a rudimentary condition in the normal flower accompanied the condition of most excessive vegetative growth. As a matter of fact, these filiform runnerlike stems represent extreme attenuations of aerial stem growth bearing the

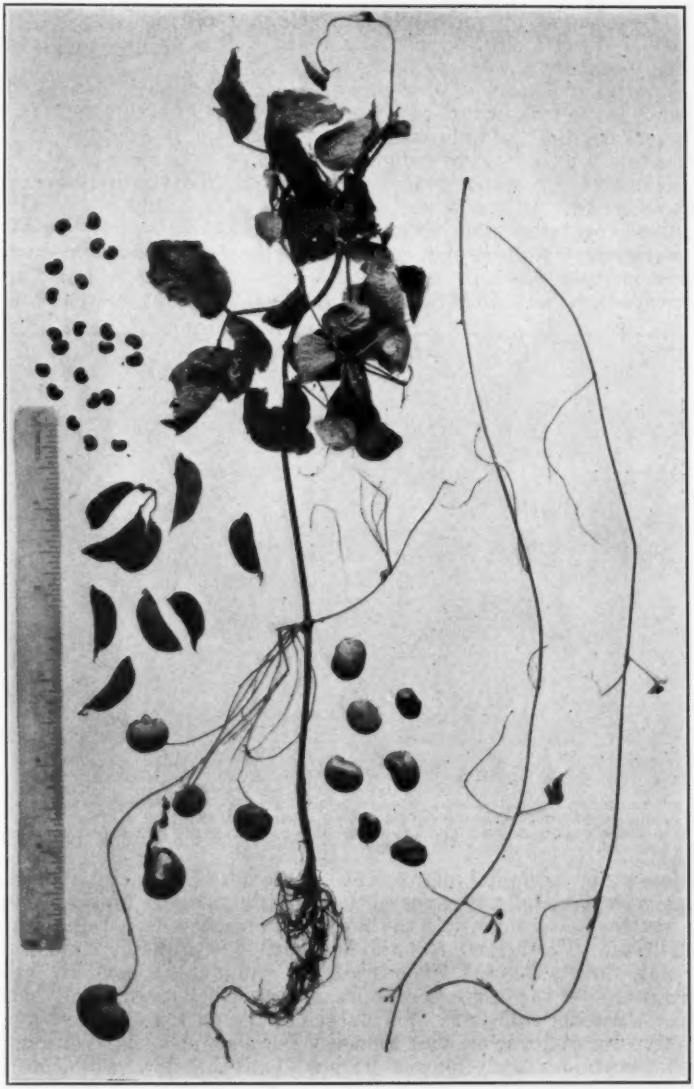

FIGURE 1.—Hog peanut grown under a 10-hour day, beginning April 28. Only greenish aerial flowers and filiform stems bearing hypogean flowers were produced. Closed flowers appeared June 10. Filiform stems appeared somewhat earlier. Aerial pods and small seeds are shown, and likewise the hypogean pods with their single large fleshy seeds, some of them still attached to the underground runners. The slender stems at the right represent runners from aerial leaf axils and show the extreme reduction of the leaves and the minute rudimentary flowers at the tips. (Photographed August 10. About one-half natural size)

inflorescence, and the leaves are greatly reduced until oftentimes they are represented by mere stipules. Upon these excessively active growing floral stems, which may reach 6 feet or more in length, usually solitary flowers are borne at the tips.

A striking feature of this phase of sexual expression in the hog peanut is the localization of extremely vigorous growth energy in the runners bearing the hypogean type of flowers. It would seem that the axillary branches are suddenly stimulated to become more or less specialized inflorescence branches given over almost entirely to sexual reproduction. It is now well known that when many plants with a distinct vegetative stem, as in the case of the cosmos, are forced into flowering by suddenly reducing the length of day, the reproductive cycle is established by a marked elongation of the terminal inflorescence stem and branches. In these plants, however, with a more

FIGURE 2.—Hog peanut grown under different day-length treatments. Tests began with wild plants taken from the field grown from naturally sown hypogean seed May 6; photographed June 18. Left to right: Controls, experiencing the full summer day, 12-hour day, 13-hour day, 13½-hour day, darkened from 10 a. m. to 2 p. m. each day. The greater abundance and length of the filiform stems bearing the hypogean type of flower is well shown on the plants exposed to a 13-hour and a 13½-hour day. At this time the controls showed no flowers of any form whatever

or less stricly terminal inflorescence, although there may be a reduction of the true foliage leaves to the condition of mere bracts, there is no noticeable reduction of the flowers themselves to a rudimentary condition. The behavior of the hog peanut is probably comparable to this, since a favorable length of day stimulates a similar marked elongation of the inflorescence branches, which arise from nearly all of the older leaf axils. So marked is the response toward reproductive vigor, however, that the weak floral stems develop extremely long internodes and become leafless, and the flowers themselves undergo extreme degeneration to the merest rudiments in comparison with the normal perfect showy blossom.

Coincident with the rapid growth of the axillary filiform stems bearing the reduced cleistogamic flowers, the hypogean pods develop with great rapidity. It is evident that the plants have transferred their energies to these long reproductive stems, with a rapid mobilization of material in all those beans that have a hypogean environ-

ment. This very rapid growth of the axillary stems producing the hypogean type of flowers does not exert any unfavorable action upon the fertility of the hypogean beans, for these are usually more viable than the small aerial beans and from the outset produce larger and more vigorous plants. This behavior of the hog peanut is similar to that of a number of other plants that regularly produce conspicuous or chasmogamic flowers and cleistogamic flowers. In the case of *Polygala polygama* Walt. and *P. pauciflora* Willd. the chasmogamic flowers are very uncertain in the production of seed, while the cleistogamic flowers produce seed in abundance.

FIGURE 3.—Winter-grown plants of the hog peanut obtained from seed of aerial legumes sown October 31, 1930, in the greenhouse, germinated November 30, and photographed January 26, 1931. Plants at right were exposed to light turned on each day from sunset till midnight. The controls at left experienced the normal seasonal length of day and produced the hypogean type of cleistogamic flower. Aerial flowers of the greenish cleistogamic winter type which developed pods were also in evidence January 8

RESPONSE OF THE PLANTS TO ARTIFICIAL LIGHT IN WINTER

Experiments have been carried out during the winter with artificial light. (Figs. 3 and 4.) The plants were grown from the small dry seeds of aerial pods which were sown October 31, 1930, and germinated November 30. Four plants were grown in a bucket. Beginning December 17, a 100-watt light with reflector 1 foot above the plants was kept on from sunset until midnight each day. These plants were stimulated to grow rather vigorously, and became twiners. A few weak filiform stems were in evidence December 19, bearing the rudimentary hypogean type of cleistogamic blossom, but none appeared to produce seed. At no time was there any evidence of aerial flowers, both the greenish winter type of cleistogamic flower and the perfect bluish blossoms being entirely suppressed.

The controls experiencing the normal winter day throughout the experiment developed filiform stems bearing the reduced hypogean type of flower. In addition a number of aerial flowers of the greenish

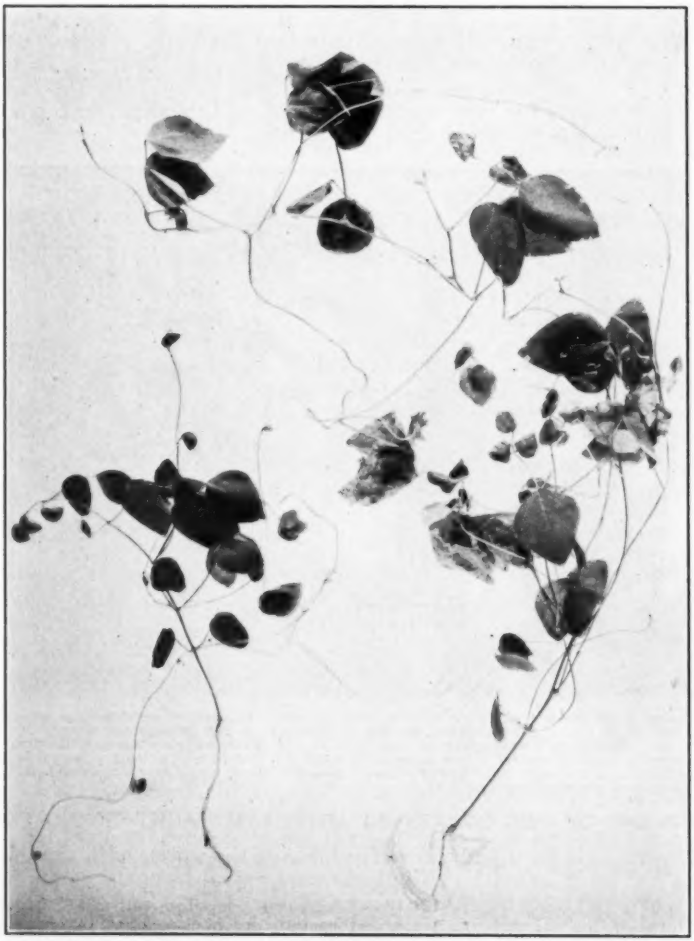

FIGURE 4.—Plants of the hog peanut of the winter type, grown from aerial seed. The plant at the right was grown with the addition of artificial light each day. Only filiform type of stem bearing the hypogean flowers had developed, all aerial forms of blossoms being suppressed. No pods had developed on these filiform stems when photographed January 26, 1931. At the left is a control plant grown without the addition of artificial light to supplement the winter length of day, with pods developed from aerial cleistogamic flowers. These pods are small, orbicular, 1-seeded, and appear to represent a nearer approach to the hypogean type of pod than the pods developed from aerial cleistogamic or chasmogamic flowers in summer. Filiform stems bearing the typical hypogean type of pod are also shown

cleistogamic type were produced, from which developed the small orbicular, mostly 1-seeded type of aerial pods characteristic of winter-grown plants.

The weak development of filiform runners on the plants subjected to additional electric light at sunset appears to have resulted from the delay in beginning the experiment. The plants had grown 17 days from germination before the normal length of day had been extended by electric light. It would appear that the additional light had stimulated the plants grown from aerial seed to greater vigor, comparable to that shown at the outset by plants grown from hypogean beans.

In a second test hypogean beans were dug from the soil in the writer's garden and planted in pots January 19, 1931. On January 30, when these had come up, one bucket containing six plants was placed beneath a 100-watt light, which was kept on from sunset till midnight. Axillary filiform stems did not develop, but instead short, thick vegetative stems appeared. The controls produced long, axillary stems, which appeared on February 11 and grew vigorously.

DISCUSSION

From the data presented it is obvious that, other conditions being uniform, flowering in the hog peanut may be very largely controlled by the length of the day to which the plants are exposed. It would appear that in the field the usual sequences of flowering with respect to the type of blossoms produced are controlled more or less by the normal seasonal changes in the length of day from spring until autumn. Experiments with constant lengths of day indicate that filiform stems bearing rudimentary hypogean flowers are stimulated to appear under day lengths as low as 5 hours, and continue to develop through an increasing series up to at least 13½ hours. At some point between 13½ hours and 15 hours, which is the maximum length of day in the locality of Washington, D. C., it would appear that the days may become too long for an immediate production of this type of inflorescence. This is shown by the fact that control plants did not begin to produce filiform stems until July 22, while plants exposed to a constant day length of 13½ hours began to produce them with great vigor by May 29.

The greenish cleistogamic aerial flowers may somewhat precede the blue aerial flowers, or they may appear after the latter have developed. In some of the experiments these closed aerial flowers appeared August 12, while the blue flowers appeared August 20. In the field the blue aerial flowers usually appear in late July or in August, to be replaced later in the summer by the greenish aerial cleistogamic flowers. The latter apparently represent a response to shorter lengths of day than are favorable to the production of the blue aerial type.

It appears that the hypogean beans are more abundantly produced than the aerial legumes. The former not only germinate with much more certainty but actually produce more vigorous seedlings. These facts would indicate that the perpetuation of the species is more dependent upon the hypogean seed than upon seed developed in aerial legumes. The rapidity of development of the hypogean seeds, which appears to be associated with the extremely rapid growth of the filiform runners bearing them, is an additional accomplishment of value to the species, since it allows an abundance of seed to be produced late in the season at a time when rapid development is most needed.

Since the hypogean beans can be produced under very short lengths of day, it is obvious that the plant need not depend upon the more uncertain aerial beans for reproduction, even in regions where strictly aerial flowers are not able to set seed. It is probably true that the smaller and extremely hard, dry, aerial beans are better adapted to distribution, perhaps by birds or other agencies, than the larger, softer, self-planted terrestrial beans. From the fact that these can develop under very short lengths of day, even as low as five hours, it is obvious that the capacity to produce hypogean beans alone would favor the natural distribution of the hog peanut into lower latitudes far southward. As a matter of fact, its known distribution extends from New Brunswick and Minnesota to Nebraska and southward to Florida and the Gulf States.

It may be remarked that the flowering behavior of the hog peanut is quite comparable to that of the cultivated peanut (*Arachis hypogaea* L.). In the case of the latter, however, only those blossoms that can push themselves under the soil produce hypogean legumes or pods. Showy aerial flowers may arise from the higher leaf axils, but these never produce aerial pods as in the case of the hog peanut.

A number of familiar wild plants have a common habit of producing cleistogamic flowers with a more or less definite seasonal incidence. Among these are Venus lookingglass (*Specularia perfoliata* (L.) A. DC.), many violets, certain species of Oxalis, which regularly develop special subterranean stems late in summer, *Polygala polygama*, and the beautiful *P. pauciflora*. Beechdrops (*Leptamnium virginianum* (L.) Rab.) likewise develop chasmogamic and cleistogamic flowers at rather definite times during the season. In practically all the plants mentioned, the conspicuous or chasmogamic flowers may be entirely sterile or produce seed only occasionally. As a rule the cleistogamic flowers are highly fertile.

As in the case of the hog peanut, it will probably be found that the behavior of many wild plants such as those mentioned, expressing itself in the production of several types of flowers, varying from the conspicuous chasmogamic forms to the extremely reduced cleistogamic forms, represents definite responses to varying lengths of day. To say the least, length of day should now be looked upon as one of the seasonal factors which can in some instances exert a profound influence not only upon the initiation of flowering itself but upon the type of flower and inflorescence produced.

SUMMARY

Experiments have been carried out with the hog peanut (*Falcata comosa* (L.) Kuntze), giving it regulated exposures to daylight by means of suitable dark houses to exclude the daylight of early morning and late evening. Response to these conditions indicates that the blue aerial perfect flowers can develop only when the days are not less than 13½ hours long. The greenish aerial cleistogamic flowers can develop under all day lengths ranging from 5 to 13½ hours. Control plants (exposed for full day) usually did not develop any form of aerial flower until late July or August. The extremely rudimentary cleistogamic type of flower borne on the slender, filiform, nearly leafless stems and giving rise to hypogean pods was able to develop under all lengths of day from 5 hours up to 13½ hours. It is evident that the upper limit inhibiting this development lies

somewhere between 13½ and 15 hours, the maximum length of day of the Washington region. This is indicated by the fact that control plants did not produce these hypogean flowers until late July or early August.

The use of weak electric light to supplement the short days of winter from sunset until midnight inhibited the development of the winter form of aerial cleistogamic flowers. It did not entirely inhibit the development of filiform stems bearing the extremely rudimentary hypogean flowers when tne additional light was withheld until 17 days after germination. In this instance no hypogean beans developed, and the filiform stems finally died. In a later test, when the additional light was afforded the plants from germination, the development of filiform stems was entirely inhibited.

In the field length of day seems to operate in fixing the more or less regular seasonal incidence of the several forms which appear to be derived from the normal blue flowers of the species, by gradations of reduction of the floral structures until the extreme hypogean type is attained.

These responses under controlled conditions indicate that the seasonal factor of length of day must be considered a potent influence in determining the kind of flowers that the hog peanut will produce. While the form of the hypogean bean is determined entirely by specific conditions associated with a soil environment, a specific length of day determines the particular type of axillary stem growth and geotropic behavior that will allow the rudimentary flowers to attain this particular environment.

THE DOWNY SPOT DISEASE OF PECANS[1]

By J. B. DEMAREE, *Pathologist*, and J. R. COLE, *Associate Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In 1927 Boyd[2] reported observing in southern Georgia a new foliage disease of the pecan (*Hicoria pecan* Brit.) and suspected that the causal fungus was a species of either Cylindrosporium or Cercosporella. The same year Demaree and Cole[3] published a short description of the disease and considered the causal organism identical with *Cylindrosporium caryigenum* Ell. and Ev. This fungus was first reported by Ellis and Everhart[4] from specimens of *H. cordiformis* (Wang.) Brit. (*Carya amara* Nutt.) collected by John Dearness near London, Ontario, in 1889. The fungus was later transferred to the form genus Cercosporella by Höhnel.[5] The discovery of the perfect form, recorded in this paper, has compelled a change of the generic name, the name of the fungus now becoming, as will be shown, *Mycosphaerella caryigena* (Ell. and Ev.) n. comb.

The disease was not noticeably abundant in southern pecan orchards in 1926, but the following year it became epiphytotic in restricted areas in southern Georgia and northern Florida. In 1928 it was less widely distributed, but probably was more abundant than in 1926. Because the disease is not a conspicuous one and is not especially destructive in Georgia, it may have been present there in pecan orchards for a number of years without being recognized as a distinct disease. Since 1926 it has been found over a large area in the southeastern part of the United States, including portions of Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Texas, and furthermore it has been found to be more destructive in restricted localities of the drier regions of Louisiana and Texas than elsewhere.

ECONOMIC IMPORTANCE

Since the disease has been under observation only a comparatively short time, its future economic importance can only be conjectured. There was a marked deficiency of rainfall in southern Georgia during the season of 1927, the year of the greatest observed prevalence of the disease, and future observations may show some association between its prevalence and a weakened condition of the trees caused by a deficiency of soil moisture, soil fertility, etc.

Observations made so far indicate that the disease may be a difficult one to control should it later prove to be destructive. It devel-

[1] Received for publication July 29, 1931; issued March, 1932.

[2] BOYD, O. C. PROGRESS REPORT ON THE EXPERIMENTS IN THE CONTROL OF PECAN SCAB AND LEAF CASEBEARER AND ON THE OCCURRENCE OF AN UNDESCRIBED LEAF-SPOT OF PECANS. Natl. Pecan Growers Assoc. Proc. 26: 30-47. 1927.

[3] DEMAREE, J. B., and COLE, J. R. TWO UNREPORTED LEAF SPOTS OF PECAN. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11: 135-136. 1927. [Mimeographed.]

[4] ELLIS, J. B., and EVERHART, B. M. NEW SPECIES OF NORTH AMERICAN FUNGI FROM VARIOUS LOCALITIES. Phila. Acad. Nat. Sci. Proc. 1893: 168. 1894.

[5] HÖHNEL, F. BEITRAG ZUR KENNTNIS DER GATTUNG CYLINDROSPORIUM GREV. Ann. Mycol. 22: 199. 1924.

oped unabated in 1927, 1928, and 1929 in orchards where four and six applications of 20 per cent monohydrated copper sulphate and 80 per cent lime dust were used for the control of pecan scab. As far as the writers are aware, no experiments have been conducted with the primary object of controlling the disease, but observations on control have been made in spraying experiments conducted for the control of other pecan diseases.

FIGURE 1.—White spots on the dorsal surface of a pecan leaflet, formed by the conidia of the downy-spot fungus

The fungus invasion does not kill the affected leaf tissues at first, but apparently it does destroy the chlorophyll in the affected areas, judging from the change of color from green to yellow. Consequently, numerous infections undoubtedly result in reduced photosynthesis. The disease has never been known to cause defoliation during the summer, but badly affected leaves ordinarily fall earlier than healthy ones.

There is some difference in the susceptibility of pecan varieties to parasitism by the fungus. The Delmas variety has shown greater susceptibility than others. This variety, however, formerly much planted, is not now considered commercially valuable within the region in which downy spot has been found, on account of its susceptibility to another pecan disease, pecan scab. The Moneymaker and Stuart, both important commercial varieties, rank next in suceptibility to downy spot. Of the other widely planted varieties the Frotscher has exhibited moderate susceptibility, while Schley, Alley, Success, and Pabst seem to be quite resistant.

DESCRIPTION OF THE DISEASE

The disease first appears on young pecan leaves during late spring or early summer. The first manifestation is the appearance of small, faintly marked, whitish spots on the underside of the leaves. These spots are made up of numerous minute clusters of conidia issuing from the stomata. The conidia are produced in great numbers from each stomatal opening on affected areas, and as the clusters enlarge they unite, forming distinct white spots from 2 to 5 mm in diameter. (Fig. 1.) The action of dew sometimes spreads the conidia over or to one side of the affected host tissues as a thin white layer. Heavy rains wash away the conidia, leaving only slight evidence of the affected spots. At first the host tissues show little or no discoloration as a result of the para-

sitism. As the infections become older the affected tissues turn yellow or light brown and are discernible also on the upper side of the leaves. The signs of the disease are never especially conspicuous after the conidia disappear. The white spots, while not permanent, furnish the most striking and descriptive sign of the presence of the pathogene.

On account of the yellow spots formed by the invasion of this Cercosporella, Boyd gave to the disease the name yellow leaf spot. Since the puncture of the pecan black aphid (*Myzocallis fumipennellus* Fitch) causes an even more conspicuous yellow spotting of pecan leaves, the writers feel that the name yellow leaf spot would frequently be confused with the effect of the aphid punctures, and therefore they suggest the name downy spot disease.

THE CAUSAL FUNGUS

CONIDIAL STAGE

The mycelium, although abundant, is confined principally to the spongy parenchyma tissues, but penetrates slightly into the palisade region. The hyphae seem to be intercellular only. During the early stage of the development of the fungus, subepidermal stromata are formed immediately below stomatal openings lying within infected areas. From the subepidermal stromata strands of hyphae extend through the stomatal openings (fig. 2, A) and form ectodermal stromata, from which numerous conidia are produced. There is some evidence that decumbent hyphae are formed from the outer stromata, from which conidia are also produced. The conidia are hyaline, mostly sickle shaped, pointed at the apex, and 2 to 3 septate. (Fig. 2, B.) Definite conidiophores have not been demonstrated. It is certain, however, that if the conidia are not sessile they must be formed on very short stalks.

Through the kindness of John Dearness, a cotype collection of *Cylindrosporium caryigenum* was examined by the writers. The conidia of the Canadian hickory specimens are formed from tufts of hyphae issuing from the stomata as described above for the form on the pecan, and there is no morphological difference between the conidia of the two forms except that those on the hickory are slightly smaller. Ellis and Everhart reported dimensions of conidia in the Dearness collection as 25μ to 40μ by 3μ, and Höhnel reported them as 25μ to 46μ by 2μ to 3μ. Conidia from the pecan range from 25μ to 55μ by 4μ to 7μ, being slightly longer and wider than those on the hickory.

PYCNIDIAL STAGE

During late summer small conical pycnidiumlike structures (fig. 2, D), 43μ to 50μ wide, form on the affected tissues that produced conidia earlier in the summer. These bodies are at first subepidermal and brown, but turn very dark brown or black after they break through the epidermis. The body wall is two to three cells thick. These structures are filled with minute rod-shaped, sporelike bodies, which escape through either a circular pore or a narrow slit. These bodies, which may be considered as pycnospores, microconidia, or spermatia, are hyaline and nonseptate. Repeated attempts to induce them to germinate in water have given negative results.

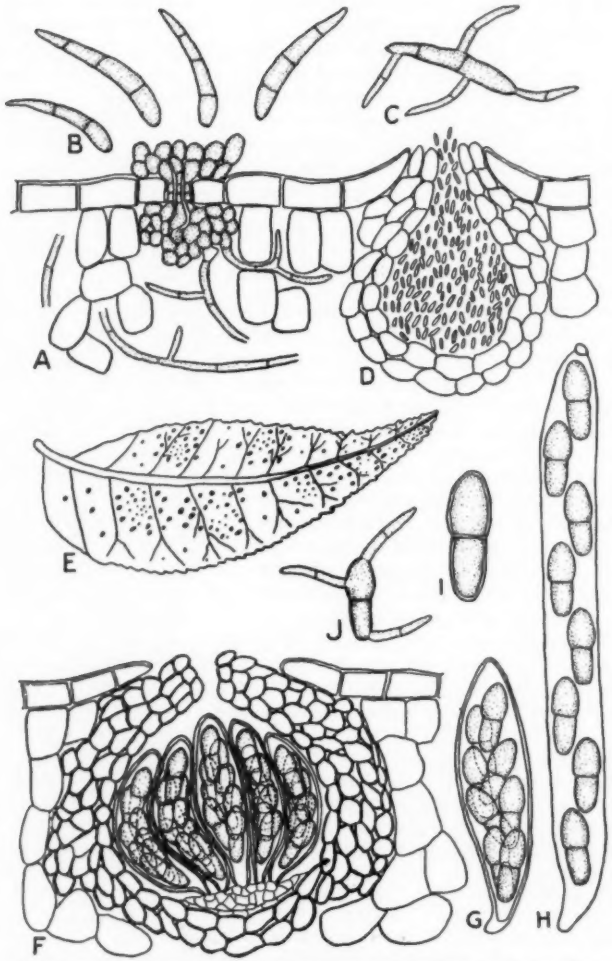

FIGURE 2.—Conidial and ascigerous stage of the pecan downy spot fungus, *Mycosphaerella caryigena*. A, Cross section of pecan leaflet showing stromata above and and below a stoma connected by hyphae; × 500. B, Conidia; × 440. C, Germinating conidium; × 440. D, Pycnidium containing sporelike bodies; × 500. E, Pecan leaflet showing distribution of perithecia; × ½. F, Cross section of a mature perithecium; × 650. G, Greatly enlarged ascus; × 800. H, Ascus showing shape and arrangement of spores after elongation; × 800. I, Enlarged ascospore; × 1,500. J, Germinated ascospore: × 850

PERITHECIAL STAGE

Primordia of perithecia develop among the pycnidia during the latter half of the summer or early fall. These bodies are at first similar to the pycnidia in color and shape, but are larger and have either a flat or a rounded top.

The perithecia may develop in groups or in irregular rings surrounding the infected host tissues that previously produced Cercosporella conidia. (Fig. 2, E.) Those formed on the periphery of areas that produced conidia are larger than those found directly upon the lesions. If numerous infections of the Cercosporella stage appeared on the leaves during the previous summer, the perithecia develop more or less uniformly over the entire lower surface of the affected leaves. The primordium of the perithecium appears first as an undifferentiated mass of hyaline fungus cells devoid of any evidence of a perithecial wall. The perithecial wall, which is formed during a later stage in the development of the fruiting body, is composed of several layers of thick-walled cells. The ostiole is a mere opening without any suggestion of a beak. (Fig. 2, F.) Mature asci have been found on fallen leaves during March and April in orchards in southern Georgia, but asci developed more rapidly on leaves that were brought into the laboratory during early winter and placed in moist chambers.

The asci are hyaline, short, thick, and slightly clavate, and are packed closely within the perithecium. When removed from the perithecium and placed in water for microscopic examination they frequently elongate to twice their original length, or more. (Fig. 2, H.) Prior to this elongation of the asci the eight spores are packed closely within the confines of the ascus wall (fig. 2, G), but after elongation the spores rearrange themselves so as to occupy uniformly the increased space formed. The spores are liberated through a pore at the apex of the ascus after elongation of the ascus takes place. They are hyaline, 1 septate, and the two cells are slightly unequal in size. (Fig. 2, I.) The smaller cell of the two points toward the base of the ascus.

PATHOGENICITY AND RELATIONSHIP OF THE DIFFERENT FORMS

The discovery of perithecia and ascospores on fallen pecan leaves in districts where *Cercosporella caryigena* was prevalent during the previous summer suggested a possible relationship between the two. Primordia of perithecia were observed developing on lesions caused by *C. caryigena* during late summer prior to leaf fall. Development of these primordia was followed through to maturity by frequent macroscopic and loose-mount examinations and by the aid of stained slides made at intervals during the development of the perithecia.

During the fall of 1927 pecan leaves were collected from a locality where the downy spot disease was abundant. At the same time other leaves were collected in a different orchard where the disease was not found. Both lots of leaves were kept out of doors during the winter in a coldframe covered with wire netting. When they were examined the following spring it was found that the collection of leaves bearing lesions of *Cercosporella caryigena* produced abundant perithecia of the type described herein, and those leaves not having lesions of *C. caryigena* produced no perithecia of that type.

Artificial cultures made from ascospores make a growth indistinguishable from that made from conidia of *Cercosporella caryigena*. Furthermore, similar conidia are produced in cultures made from either ascospores or conidia isolated from leaves. The pycnidia are considered to be related to the other two spore forms because of their frequent presence on lesions caused by the conidial stage, and because they are commonly produced in artificial cultures originating from either ascospores or conidia.

To obtain further evidence of the relationship of the perfect form to *Cercosporella caryigena*, inoculation experiments were made. The following series of experiments were conducted in a locality where the Cercosporella was not abundant.

1. Ascospores were collected by inverting plates of agar about 2 to 3 mm. above leaves containing mature ascospores lying on moist filter paper. The spores were discharged on the surface of the agar and allowed to germinate. Blocks of agar containing the germinating spores were removed from the Petri dishes and placed on the under surface of young pecan leaves.

2. With the aid of a binocular microscope mature perithecia were removed from the leaf tissues, crushed, and placed in a watch glass containing about 10 c. c. of distilled water. A medicine dropper was used to place the inoculum suspension on the under side of young leaflets.

3. Conidia taken from the successful inoculations of Nos. 1 and 2 were planted on agar plates and allowed to germinate. Blocks of agar with the germinating conidia were placed on young leaflets of the Moneymaker variety.

4. Conidia in water suspensions were applied to the under surface of young leaves with a medicine dropper.

One hundred inoculations were made in each series. Inoculated leaflets were inclosed in paraffin paper bags for a period of 24 hours. All inoculated leaflets were tagged and marked with powdered white crayon. No natural infection was found in experimental trees until after August 1, and not more than 1 per cent of noninoculated leaves were infected after that date. The results are set forth in Table 1.

TABLE 1.—*Results of inoculation of pecan leaflets with conidia of Cercosporella caryigena and the associated perfect stage* [a]

Inoculum	Date of inoculation	Date record taken	Number of inoculations	Number of infections
Germinated ascospores in cubes of agar	Apr. 28	June 16	100	31
Do	May 7	June 25	100	37
Macerated perithecia in water suspension	...do	...do	100	5
Germinated ascospores in cubes of agar	June 1	July 10	100	55
Do	June 10	July 20	100	43
Macerated perithecia in water suspension	...do	...do	100	10
Germinated conidia in cubes of agar, originating from infections by ascospores	July 15	Aug. 20	100	63
Do	July 20	Aug. 30	100	30
Conidia in water suspension, taken from successful ascospore inoculations	Aug. 1	Sept. 15	100	0

[a] The inoculations reported were performed by the junior writer near Shreveport, La.

Of the 600 ascosporic inoculations made, 181 resulted in infections typical of *Cercosporella caryigena*. A period of six to seven weeks

was required from the time the inoculum was applied to the leaves until Cercosporella conidia were produced. Conidia that developed from ascosporic inoculations were used as the inoculum for subsequent experiments, which in turn produced Cercosporella conidia. The largest percentage of successful inoculations resulted from germinated ascospores and conidia in agar. Inoculations from macerated perithecia gave a low percentage of infection, but no infections resulted when the inoculum consisted of conidia in water suspension.

As a result of the cultural and inoculation experiments, the relationship of the Cercosporella stage and the Mycosphaerella stage is proved, and both are shown to be pathogenic.

TAXONOMIC POSITION AND TECHNICAL DESCRIPTION

The characters of the ascigerous stage of this fungus correspond to those of the genus Mycosphaerella as described by Johanson, to which genus this fungus is assigned. Apparently there has not been described a Mycosphaerella on the pecan or other species of Hicoria that is morphologically identical with this one; and since it has proved to be connected with *Cercosporella caryigena* (Ell. and Ev.) Höhnel, it may appropriately be given the name *Mycosphaerella caryigena* with the following description:

Mycosphaerella caryigena, n. comb.

SYNONYMS: *Cylindrosporium caryigenum* Ellis and Everhart. Proc. Acad. Nat. Sci., Phil., Pa., 1889.

Cercosporella caryigena (Ell. and Ev.) Höhnel, Ann. Mycol., vol. 22., 1924.

ASCIGEROUS STAGE.—Perithecia hypophyllous, black, erumpent, aggregate to scattered, ellipsoid-globose, 50μ to 85μ in diameter, ostiolate. Perithecial wall 2 to 5 cells thick. Asci clustered, clavate-cylindrical, hyaline, 8-spored, 45–55μ by 7.5–9μ (often becoming much longer when placed in water); no paraphyses; spores hyaline, 1-septate, slightly unequal, 12–15μ by 3–5μ.

CONIDIAL STAGE.—Hyphae principally confined to spongy parenchyma; conidia profuse, formed from tufts of hyphae penetrating through stomata; hyaline, filiform, curved, 2 to 3 septate, 25–55μ by 4–7μ. Conidia form white spots on lower leaf surface of Hicoria, later disappearing, and lesions show as moderately conspicuous yellow to light-brown spots 2 to 5 mm in diameter.

PYCNIDIAL STAGE.—Pycnidia appear during late summer on lesions caused by the Cercosporella stage, hypophyllous, first brown, later black, erumpent, 45–50μ wide. Pycnospores rod-shaped, hyaline, nonseptate, 2–3.5μ by 1–1.5μ. Function unknown.

Parasitic on leaves of Hicoria species.

Ascigerous stage first appearing on leaves of *Hicoria pecan* during late summer; development completed in March and April of following year.

PHYSIOLOGICAL STUDIES ON ARTIFICIAL MEDIA

Monosporous cultures from conidia were readily made by removing them with a sterile needle from young sporulating lesions and "streaking" agar plates. Isolated conidia were later transferred to agar slants. The perfect stage was cultured by inverting plates of agar over fragments of leaf tissue containing mature perithecia. The most satisfactory plantings were made when the perithecia were lying about 2 mm below the surface of the agar. Isolated spores were marked, and those that germinated were transferred to tubes containing agar. The cultures grew very slowly at first, requiring about eight days at room temperature (about 23 to 27° C.) to become macroscopic in size, and attained a diameter of 1 to 1½ mm in about two weeks.

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The fungus was grown on the following types of artificial culture media: Beef agar, corn-meal agar, Lima-bean agar, dextrose agar, a mixture of corn-meal and potato agar, pecan leaves, watermelon rind, and bean pods.

The reaction of the fungus on each medium used was different. On dextrose agar (Difco) it produced a dark-brown to black granular thick stroma on the surface of the medium. Little or no aerial mycelium was present. The advancing portions of the stroma had a creamy to grayish color and were somewhat slimy. The entire stroma as seen through a lens had a wet, slimy appearance. On Lima-bean agar the exposed stroma was rough or granular and dark brown. The stromata were usually covered with a mat of white hyphae, surrounded by white to creamy, slimy, submerged portions.

Cultures on most media form conidia and pycnidia in abundance. Both conidia and pycnospores closely resemble those occurring naturally on the host. Perithecia have not been known to form on artificial media.

On artificial media the fungus made the most vigorous growth at temperatures ranging from 23° to 27° C. Growth is inhibited at a temperature of about 5°, but is resumed when the temperature becomes more favorable.

The fungus was grown at the optimum temperature for 60 days on corn-meal agar, having a hydrogen-ion concentration of pH 3.8 to 8.5. The best growth was made at pH 6.5. Below pH 4.5 and above 8.0 growth was very meager.

SUMMARY

The conidial stage of the downy spot disease of pecans is considered identical with *Cercosporella caryigena* (Ell. and Ev.) Höhnel, a fungus first described by Ellis and Everhart as *Cylindrosporium caryigenum* on specimens of *Hicoria cordiformis* collected by John Dearness near London, Ontario, in 1889.

Conidia are produced in great numbers on lesions and form white spots 2 to 5 mm. in diameter on the lower surface of affected leaves. When the conidia are washed off by rains the lesions appear as inconspicuous yellow or brown spots. The disease has been observed on the pecan in Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Texas.

The perfect stage, *Mycosphaerella caryigena* n. comb., develops on pecan leaves during fall and winter, but does not mature until early spring. Proof of the relationship of the conidial and perfect stages and of their pathogenicity was demonstrated by comparison on artificial media and by inoculation experiments.

A description of the fungus and its growth on culture media is given.

PHYSIOLOGIC RACES OF USTILAGO LEVIS AND U. AVENAE ON RED OATS[1]

By GEORGE M. REED, *Curator, Brooklyn Botanic Garden,* and T. R. STANTON, *Senior Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The results of a study of smut specimens for the determination of specialized races of *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn. are presented in the following pages. This paper also reports the identification of a hitherto unknown physiologic race of *U. levis* which attacks Fulghum oats.

REVIEW OF LITERATURE

Extensive data on physiologic races of the fungi that cause loose and covered smuts on oats have been published by Reed (*4, 5, 6*)[2] and by Sampson (*7, 8*). Several distinct races of both species have been identified. Among the races of *Ustilago avenae* two of particular interest attack the Fulghum and Red Rustproof varieties. The Fulghum race of *U. avenae* is capable of severely smutting Fulghum and the closely related Kanota and Frazier strains. In addition, several varieties of common oats, such as Bicknell, Black Diamond, Canadian, and Victor, are severely attacked. Hull-less or naked oats and the wild species *Avena barbata* Brot. also are very susceptible. On the other hand, strains of the Red Rustproof type are extremely resistant to this particular race of *U. avenae.*

The physiologic race of *Ustilago avenae* which attacks Red Rustproof also is highly specialized. It produces a large percentage of smutted plants on Red Rustproof and the related Nortex. On Fulghum and its strains, however, it gives essentially negative results. Apparently there is only one variety of the *Avena sativa* group, namely Canadian, that is susceptible. Hull-less oats also are extremely resistant. It is an interesting fact, however, that *A. barbata* is completely susceptible.

Hitherto no race of *Ustilago levis* has been known to attack seriously Fulghum and Red Rustproof oats. Reed (*6*) has shown that Fulghum occasionally may be slightly smutted by a form of *U. levis* from Missouri, but the percentage of smutted plants is never very high. Occasional plants of Red Rustproof, apparently smutted with this same race, also have been found.

IMPORTANCE OF FULGHUM AND RED RUSTPROOF OATS

Variously named strains of the Fulghum and Red Rustproof varieties of oats are grown extensively in the southern half of the United

[1] Received for publication July 17, 1931; issued March, 1932. The investigations here reported were conducted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Brooklyn Botanic Garden, Brooklyn, N. Y. Contribution No. 62 Brooklyn Botanic Garden.

[2] Reference is made by number (italic) to Literature Cited, p. 152.

States. In the Cotton Belt they are grown almost exclusively, and mostly from fall seeding. In the region immediately to the north of the Cotton Belt they are important spring-sown varieties, especially Fulghum and its strain Kanota. Fulghum is now most extensively grown in Missouri, Kansas, and Oklahoma, where it has largely replaced the Burt and Red Rustproof varieties. The last named is not altogether satisfactory for spring seeding, owing to its later maturity. Prior to the development of Fulghum, early varieties of common oats such as Kherson and Sixty-Day also were grown to some extent in this area, with generally unsatisfactory results, owing to poor adaptation. Stanton and Coffman (*9*, *10*) have made available information on the importance and distribution of the Fulghum and Red Rustproof varieties.

MATERIALS AND METHODS

In the spring of 1929 the junior writer collected samples of oat smut in various parts of the South and forwarded them to the senior writer at the Brooklyn Botanic Garden. An identification number was assigned to each collection, by which each is hereafter designated. Ten of these collections have been tested for their physiologic specialization. The oat varieties used as testers or differential hosts were Canadian (seed No.[3] 119), Victor (seed No. 126), Fulghum (seed No. 129), Red Rustproof (seed No. 131), and Navarro (Ferguson Navarro; seed No. 939, C. I.[4] No. 966).

The inoculations were made by the method described by Reed (*6*). All tests were made under greenhouse conditions at the Brooklyn Botanic Garden.

EXPERIMENTAL DATA

The reaction of the five differential hosts to *Ustilago levis* collected from the Fulghum variety, is shown in Table 1. Collection numbers and geographic origin are likewise shown.

TABLE 1.—*Reaction of the five differential hosts to Ustilago levis collected from Fulghum oats*

Variety	Collection No. 16; Athens, Ga.			Collection No. 18; Clemson College, S. C.		
	Plants	Infected		Plants	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Canadian	13	13	100.0	17	17	100.0
Victor	19	16	84.2	19	19	100.0
Fulghum	19	18	94.7	19	15	78.9
Red Rustproof	19	0	0	18	0	0
Navarro	19	0	0	19	0	0

Collections Nos. 16 and 18 proved to be typical of the covered smut. Both produced high percentages of smut on Fulghum. Collection No. 16 produced smut on 18 of the 19 plants, or 94.7 per cent, and collection No. 18 produced smut on 15 of 19 plants, or 78.9 per cent. Collection No. 16 gave 100 per cent infection on Canadian and 84.2

[3] Seed numbers designate special strains of the varieties propagated and maintained by the senior writer.
[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

per cent on Victor, while collection No. 18 produced 100 per cent infection on both of these varieties. Red Rustproof and Navarro proved highly resistant to both collections.

The reaction of the five differential hosts to *Ustilago avenae* collected from the Norton, Kanota, and Frazier varieties (collections Nos. 8, 48, and 56, respectively) is shown in Table 2. The geographic origin of each collection is also shown.

TABLE 2.—*Reaction of the five differential hosts to Ustilago avenae collected from Norton, Kanota, and Frazier oats*

Variety	Collection No. 8; A. and M. College, Miss.			Collection No. 48; Near Newton, Kan.			Collection No. 56; Lawton, Okla.		
	Plants	Infected		Plants	Infected		Plants	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Canadian	19	19	100.0	19	17	89.4	20	20	100.0
Victor	20	8	40.0	20	11	55.0	18	17	94.4
Fulghum	20	20	100.0	20	20	100.0	20	20	100.0
Red Rustproof	19	0	0	19	0	0	20	0	0
Navarro	20	0	0	20	0	0	20	0	0

Collection Nos. 8, 48, and 56 (Table 2) correspond quite closely in reaction to the Fulghum race of *Ustilago levis* shown in Table 1. All three collections gave 100 per cent infection on the Fulghum variety. Very high percentages of smut also were obtained on Canadian. The Victor variety gave somewhat variable results, the percentage of smutted plants ranging from 40.0 to 94.4. The results with Red Rustproof and Navarro were entirely negative. These data are in harmony with those reported by Reed (*5*, *6*) for the physiologic race of *U. avenae* on Fulghum oats.

The reaction of the five differential hosts to *Ustilago avenae* collected from the Ferguson No. 922, Nicholson Hundred Bushel, and Nortex strains of the Red Rustproof variety (collections 25, 53, and 57, respectively) is shown in Table 3. The geographic origin of each collection is given in the table.

TABLE 3.—*Reaction of the five differential hosts to Ustilago avenae collected from Ferguson No. 922, Nicholson Hundred Bushel, and Nortex oats*

Variety	Collection No. 25; Denton, Tex.			Collection No. 53; Stillwater, Okla.			Collection No. 57; Lawton, Okla.		
	Plants	Infected		Plants	Infected		Plants	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Canadian	19	16	84.2	20	19	95.0	19	17	89.4
Victor				20	2	10.0	20	1	5.0
Fulghum	20	0	0	20	0	0	20	6	30.0
Red Rustproof	20	15	75.0	20	14	70.0	20	19	95.0
Navarro	20	0	0	19	0	0	19	0	0

Collections Nos. 25, 53, and 57 are very similar to, if not identical with, the previously described form of *Ustilago avenae* obtained from Red Rustproof oats (*5*, *6*). The three named strains of Red Rustproof from which the collections were made are typical of the Red

Rustproof variety. Comparatively high percentages of smutted plants were obtained with all three collections on Red Rustproof, the percentages ranging from 70.0 to 95.0. A somewhat higher percentage of smut (from 84.2 to 95.0 per cent) was secured with the variety Canadian. A few smutted plants of Victor were obtained with two of the collections, but no smutted plants whatever were obtained on Navarro. Fulghum gave negative results with two of the collections, but with No. 57, 30 per cent of the plants, or 6 out of 20, were smutted. There is a possibility in this case that the original collection of smut was a mixture of the Red Rustproof and Fulghum races. Further experiments are necessary to determine whether this is the case.

Two additional collections from Cowra No. 22 and Colburt (C. I. No. 2019) also have given interesting results. The reaction of the five differential hosts to *Ustilago avenae* collected from these varieties (collections Nos. 46 and 59, respectively) is shown in Table 4. The geographic sources of the collections are shown in the table.

TABLE 4.—*Reaction of the five differential hosts to Ustilago avenae collected from Cowra No. 22 and Colburt oats*

Variety	Collection No. 46; Experiment, Ga.			Collection No. 59; Lawton, Okla.		
	Plants	Infected		Plants	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Canadian	19	19	100	19	19	100
Victor	19	19	100	19	19	100
Fulghum	20	3	15	20	0	0
Red Rustproof	20	0	0	20	0	0
Navarro	20	0	0	20	0	0

Collection No. 46, from the Cowra No. 22 variety, gave 100 per cent infection on both Canadian and Victor. The Fulghum variety showed 15 per cent of smutted plants, 3 out of a total of 20 being smutted. Negative results were secured with Red Rustproof and Navarro. In its ability to infect Fulghum slightly and to infect Canadian and Victor heavily, this collection is allied to the Missouri race of *Ustilago avenae*. The remaining collection, No. 59, gave 100 per cent infection on the two varieties, Canadian and Victor, while entirely negative results were secured with Fulghum, Red Rustproof, and Navarro.

DISCUSSION

The most interesting feature of the results of this study is the identification of a definite specialized race of *Ustilago levis*, hitherto unknown, which attacks the Fulghum variety of oats. (Fig. 1.) The two collections, Nos. 16 and 18, are typical of this species, and both show a high degree of virulence for this type of oat. As yet no similar race of covered smut capable of infecting the Red Rustproof variety has been demonstrated. It is, however, very probable that such a form or race is actually in existence and will be identified sooner or later.

Further experiments are in progress to determine more definitely the extent of the specialization of the new collections. A large number

of varieties of oats, representing different types, have been inoculated in order to determine their susceptibility.

Some further discussion of the relationship of such varieties as Norton, Cowra No. 22, and Colburt to Red Rustproof and Fulghum in connection with their reaction to certain of the 10 smut collections reported in this paper seems desirable.

It was to be expected that Norton would show high susceptibility to *Ustilago avenae* collected from Fulghum. Norton was originated as a selection from a cross between Fulghum (Coker Fulghum strain No. 3) and an unnamed gray oat (R–F–3) with side panicle, which was obtained as an individual plant from a field of mixed Red Rust-

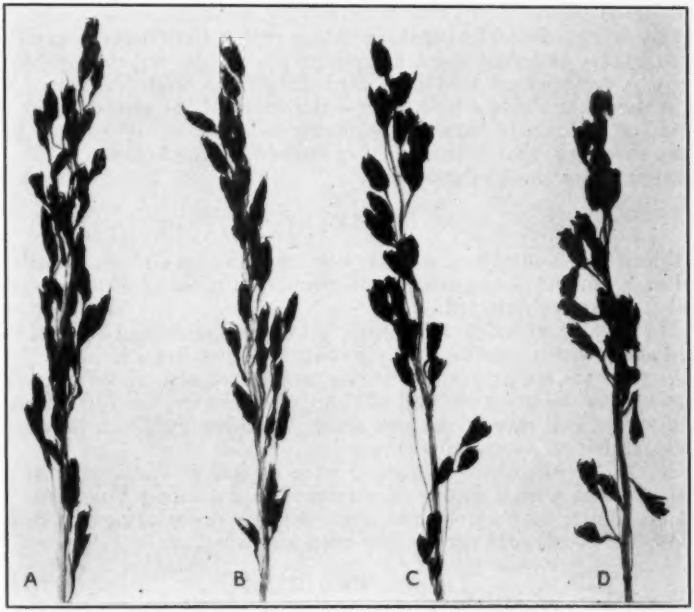


FIGURE 1.—Smutted panicles of Fulghum oats; A and B infected with *Ustilago levis*, C and D with *U. avenae*. The contrast between the two smuts is very evident

proof by George J. Wilds, jr., in 1918. The cross was made a few years later by J. B. Norton in the breeding nurseries of Coker's Pedigreed Seed Co., Hartsville, S. C.

The close relationship of Norton to the Kanota and Frazier strains of Fulghum is demonstrated by the results presented in Table 2. On the other hand, relative to plant characters, Norton resembles common rather than red oats. These facts undoubtedly furnish further evidence that the inheritance of susceptibility or resistance to smut in oats is not linked with morphological characters.

The Cowra No. 22 (also known as Quandong) variety was introduced from Australia. According to Pridham (*2*, *3*) it was originated as a selection from Ruakura on the Cowra Experiment Farm, New South Wales. The latter variety was developed from a plant varia-

tion from the Red Algerian (Argentina), a variety belonging to *Avena byzantina* C. Koch, morphologically similar to the well-known Red Rustproof oat of the South. Ruakura, however, usually has been classified as belonging to *A. sativa* L. Cowra No. 22 is similar to Ruakura in that it is more or less intermediate in type between the varieties of *A. sativa* and *A. byzantina*. In this connection it is of interest to point out that differential hosts belonging to both of these groups reacted to the smut collected on Cowra No. 22. (Table 4.)

The origin of the Colburt variety has been reported by Stanton, Griffee, and Etheridge (*11*) and by Coffman (*1*). It was developed as a plant selection from Burt, a red oat, at Akron, Colo. However, Colburt is an early black common oat (*Avena sativa*), morphologically similar to Monarch. Colburt is a very uniform variety and evidently represents a mechanical mixture rather than a plant variation from Burt. As a consequence, Colburt probably is not closely related to such varieties as Fulghum, Red Rustproof, and Navarro. The data shown in Table 4 indicate specialization of the race of smut collected on Colburt to varieties belonging to *A. sativa*. It is very probable, therefore, that this smut was introduced from Akron, Colo., to Lawton, Okla., on Colburt itself.

SUMMARY

Results of a study of a collection of smut specimens, mostly of red oats, for the determination of specialized races of *Ustilago avenae* and *U. levis* are reported.

The identification of a hitherto unknown specialized race of covered smut which attacks Fulghum oats is demonstrated.

As red oats are grown extensively and in some sections almost exclusively in the southern half of the United States, the identification of a specialized race of covered smut attacking Fulghum may be of considerable economic importance.

Of the 10 collections studied, 2 were typical of *Ustilago levis*, and both showed a high degree of virulence in attacking Fulghum. As yet no similar race of covered smut capable of infecting the closely allied Red Rustproof variety has been identified.

LITERATURE CITED

(1) Coffman, F. A.
1925. Experiments with cereals at the Akron (Colo.) field station in the 15-year period, 1908 to 1922, inclusive. U. S. Dept. Agr. Bul. 1287, 63 p., illus.

(2) Pridham, J. T.
1921. Varieties of oats tested in New South Wales. Agr. Gaz. N. S. Wales 32: 249–252.

(3) ———
1921. Descriptions of new varieties of cereals. Agr. Gaz. N. S. Wales 32: 699–703, illus.

(4) Reed, G. M.
1924. Physiologic races of oat smuts. Amer. Jour. Bot. 11: 483–492, illus.

(5) ———
1927. Further evidence of physiologic races of oat smuts. Mycologia 19: 21–28.

(6) ———
1929. New physiologic races of the oat smuts. Bul. Torrey Bot. Club 56: 449–470.

(7) SAMPSON, K.
1925. SOME INFECTION EXPERIMENTS WITH LOOSE AND COVERED SMUTS OF OATS WHICH INDICATE THE EXISTENCE IN THEM OF BIOLOGICAL SPECIES. Ann. Appl. Biol. 12: 314–325, illus.
(8) ———
1929. THE BIOLOGY OF OAT SMUTS. II. VARIETAL RESISTANCE. Ann. Appl. Biol. 16: 65–85, illus.
(9) STANTON, T. R., and COFFMAN, F. A.
1929. SPRING-SOWN RED OATS. U. S. Dept. Agr. Farmers' Bul. 1583, 18 p., illus.
(10) ——— and COFFMAN, F. A.
1930. FALL-SOWN OAT PRODUCTION. U. S. Dept. Agr. Farmers' Bul. 1640, 20 p., illus.
(11) ——— GRIFFEE, F., and ETHERIDGE, W. C.
1926. REGISTRATION OF VARIETIES AND STRAINS OF OATS. Jour. Amer. Soc. Agron. 18: 935–947.

BLACK SCORCH OF THE DATE PALM CAUSED BY THIELAVIOPSIS PARADOXA[1]

By L. J. KLOTZ, *Associate Plant Pathologist*, and H. S. FAWCETT, *Plant Pathologist, Graduate School of Tropical Agriculture and Citrus Experiment Station, University of California*

INTRODUCTION

A fungous disease of economic importance has recently appeared on the date palm, *Phoenix dactylifera*, in the Southwest. The fungus, *Thielaviopsis paradoxa* (De Seynes) Von Höhn.,[2] has been found attacking all organs of the palm except the roots and stem, and these latter organs have been found susceptible by artificial inoculation. Successful infections by means of inoculations have been obtained on all parts of the date palm, and the organism in all cases has been readily reisolated. While the total losses from this disease up to the present are apparently of minor importance, the severity of its attack in some instances indicates that it may become so troublesome as to require special measures of control.

DISTRIBUTION

In the so-called "bud-scorch" form of the malady is widely distributed, being present in every garden inspected in the Coachella Valley, Calif., and Arizona. It has also been found on ornamental date palms at Riverside, Calif. Other workers have found that the fungus parasitizes a number of plants, including areca palms, oil palms, sugarcane, coconut, and pineapple. Edgerton (*3*)[3] describes it as causing great damage to sugarcane. Although it has not been reported as occurring on Citrus, the writers have found that it produces a firm, dark, smoky-colored, pleasantly aromatic decay when introduced into wounds of citrus fruits. In India, Sundararaman, Krishnan Nayar, and Ramakrishnan (*11*) have shown experimentally that it is capable of attacking plantain, mango, *Saccharum spontaneum*, *Rhapis* sp., and the date palm. Except in the abstract by Klotz and Raby (*8*) and in papers by Fawcett (*4*) and Klotz (*7*), it is believed that the organism has never been reported as attacking the date palm naturally. On preserved specimens of apparently the same disease collected by Fawcett (*4*) in Egypt, Algeria, and Tunisia, the writers have found conidia typically like those of Thielaviopsis. How seriously the fungus attacks the inflorescences and lessens the quantity of fruit depends upon weather conditions preceding and during the time of emergence of the spathes. It is likely that mildly warm, moist weather accompanying or alternating with windy weather favors distribution and infection. The optimum temperature for growth of the fungus lies between 24° and 27½° C. The manner in which the conidia are borne (in extremely long chains, which readily break up into small groups and single conidia) favors distribution by wind. Germination of the spores on glass is possible only in the presence of water in liquid form.

[1] Received for publication Aug. 4, 1931; issued March, 1932. Paper No. 257, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Identified by J. A. Stevenson, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Reference is made by number (italic) to Literature Cited, p. 165.

SYMPTOMS OF THE DISEASE

ON SPATHE, FRUITSTALK, AND FRUIT STRANDS

As shown in Figure 1, the parasite attacks the young fruitstalks and fruit strands even before the spathe has ruptured. On the spathe, circular to elongated lesions mark the points of entrance of the disease. These lesions range in color from sorghum brown (Ridgway)[4] on the

FIGURE 1.—Female spathe cut open to show (A) black scorch on inflorescence, the gnarled and twisted fruit strands being devoid of flowers; (B) lesions on fruitstalk

exterior surface to mahogany red or bay on the interior surface. On removal of a portion of the infected spathe it was found that the fruitstalk bore depressed, brown (warm blackish) to black necrotic areas, which were circular to oblong in outline. The twisted deformed fruit strands of the specimen shown in Figure 1, A, were entirely diseased.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

They were blackish brown to black in color, and were devoid of flowers. Microscopic examination showed them to be covered by the typical dark-brown, unicellular, oval conidia. The strands of fruit bunches that were attacked later in their development showed blackened, depressed lesions (fig. 2) similar to those on the fruitstalk, and some were completely severed by the decay. The affected tissue was in all instances dry and firm and each area bore the black powdery spores. A gray covering on some of the lesions was found to be due to conidia of *Fusarium* sp. A species of Fusarium was later found as the primary cause of a decay of certain male inflorescences of the date palm. Inoculations by means of spore suspensions of Thielaviopsis nto young spathes which were just beginning to crack open showed that wounding was unnecessary for infection, the typical dark lesions being produced on the young tender fruit strands and fruitstalks.

ON TERMINAL BUD

The effect of the disease on the palm bud and heart is even more serious than it is on the fruitstalks. The pathogene gains entrance to the succulent tissue through a wound or puncture, and its progress in this vital region is very rapid. The entire terminal bud and adjacent leaf bases may succumb, eventually presenting a dried, dull, blackened, charcoallike appearance. Two large date seedlings in boxes in the greenhouse were killed by inoculations at the base of the young central leaves. In four of the five cases observed in the field the entire bud was not killed but grew out laterally, producing the so-called "fool disease" effect (called by the Arabs "medjnoon"). It is believed that in California *Thielaviopsis paradoxa* is the principal organism causing this peculiar trouble. Eventually, the entire bud regenerates from the uninjured portions of meristematic tissue and returns to its normal vertical position. High temperatures and rapid growth of the palm may be the factors that operate to prevent the disease from terminating fatally in all instances. On laboratory media the fungus makes very little growth at 32° C. or above.

ON THE PETIOLE, MIDRIB, AND PINNAE

The blackening of the midrib of fronds that usually accompanies the bud-scorch form of the disease may frequently be due to the same organism. The black, irregular, rough, necrotic condition of the leafstalk (fig. 3) is the most striking symptom of the disease. It gives the impression that the tissues have been burned, and suggests the name selected for the disease—black scorch. The cross cuts and V cuts so commonly found near the base of a midrib present an ideal entrance for this and other fungi.

Ashby (*1*) and Orian (*9*) have reported the fungus as attacking the pinnae of the freshly opened leaves of coconut palm. "Pale yellow spots with a brown margin develop on the furled pinnae. Later the lesions elongate, converge, and turn black, owing to the presence in the tissues of spores of the fungus. Infection spreads rapidly through the pinnae, and in severe cases the heart leaves dry up." This, so far as it goes, is an accurate description of the course of the disease produced by the writers on a large seedling in the greenhouse. On this seedling and on material collected in the field, the midribs and pinnae had circular to elongated irregular spots, which in some in-

stances were as wide as the pinnae and as much as 5 centimeters in length. Artificial wounding was unnecessary to secure infection on the petiole, midrib, and pinnae. The fungus readily invaded the margin of the petiole where the fibers originate. Twenty days after

FIGURE 2.—Black-scorch lesions on fruit strands. × 3¼

spores were placed on a leaf of a seedling palm, both edges of one petiole had lesions 3 to 10 millimeters in depth and 150 millimeters in length. (Fig. 4, A.) In the chlorophyll-less region of the petiole

base, the lesion was yellow ocher in color,[5] and in the green region, Dresden brown. The outer margin of the lesion was dark brown to black, while the inner margin was a light chestnut brown. The central area

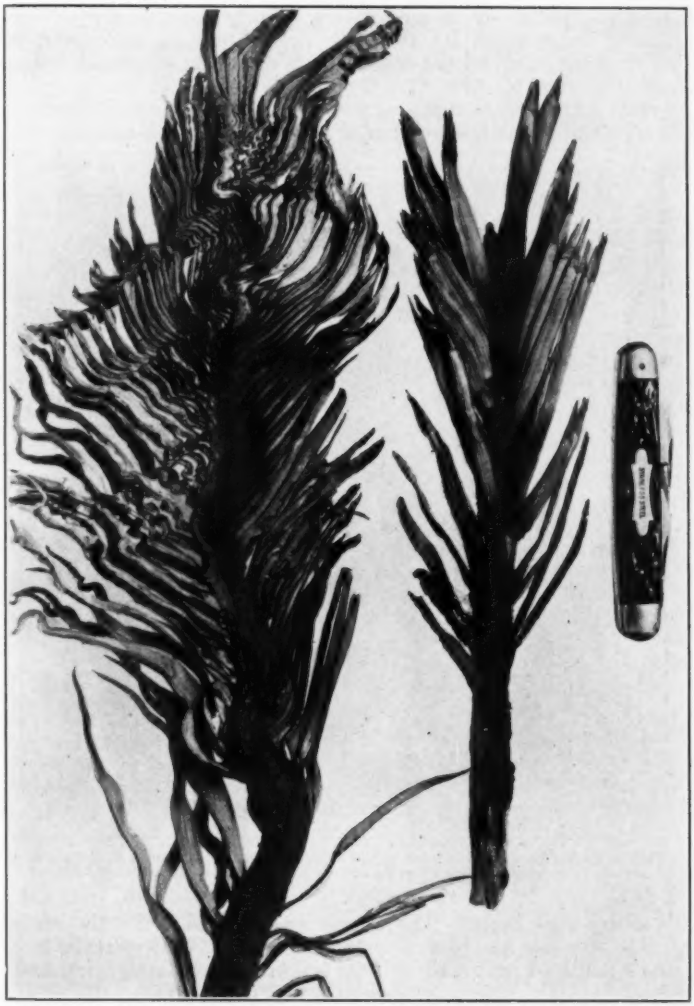


FIGURE 3.—Deformed young date fronds showing charcoal like effect that follows infection by black scorch; the fungus may accompany the deformity but does not necessarily initiate it

(17 by 10 millimeters) of a typical isolated spot (20 by 16 millimeters) on the dorsal surface of the same petiole (fig. 4, A) was chestnut brown, bounded by a narrow margin of deep brown to black. Black

[5] RIDGWAY, R. Op. cit.

spore masses were scattered throughout this area. Surrounding the inner area was a band 2 to 3 millimeters in width and yellow ocher in color. The margin of the band was a light chestnut brown. Figure 4, B, shows typical spots on the pinnae. These ranged in size from microscopic to 20 by 8 millimeters. Their color characters were similar to those given for the spot on the petiole. However, as a lesion on a pinna dries, the chestnut-brown center gradually becomes lighter until it is a warm buff.

Several midrib bases of the second whorl of fronds on a large seedling offshoot were inoculated by placing the fungus in a 3-millimeter hole

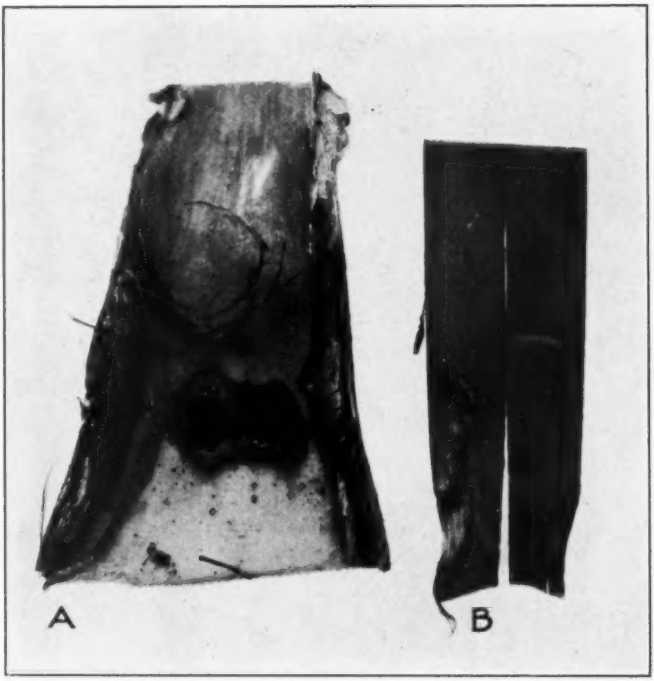


FIGURE 4.—Lesions on petiole base (A) and pinna (B) produced by fungus in absence of wound. The petiole margins where the fibers originate should be considered as wounded tissue

made with a cork borer. The wound was covered with adhesive tape until the organism had become established. In four weeks the fungus on one frond had produced an oval lesion 9 centimeters wide and 15 centimeters long, almost enveloping the midrib and causing it to break. Extending for a total length of 45 centimeters up and down the surface of the midrib beyond the lesion was a linear series of circular water-soaked areas each about 12 millimeters in diameter. The fungus was reisolated from the water-soaked area most remote from the point of inoculation, which shows that the organism invaded new tissue at the rate of at least 1½ centimeters per day for 30 days. The surface of the canker was gray to brown to drab in color, and the

pinnae beyond turned gray as they dried. Internally the lesion was light drab to wine in color toward the advancing edges, with smaller orange to reddish-brown streaks extending far up and down the midrib. (Fig. 5.) These streaks were directly under the water-soaked areas that appeared on the surface. The pathogene was readily reisolated from any portion of the affected tissue. Eventually the invaded tissue turned black owing to the production of fuscous spores by the causal fungus. Likewise, inoculations of any pruning cuts and the cut surfaces of midribs and spines were invariably successful, the infected tissue dying back several inches and eventually becoming blackened and covered by fungous spores.

ON STEM AND ROOT INDUCED BY ARTIFICIAL INOCULATION

To test the susceptibility of the trunk or stem of date palm to the fungus, the old leaf bases were cut away, the surface cleaned with alcohol, and a portion of an agar culture inserted in a hole made with a quarter-inch cork borer. The inoculation was covered with adhesive tape. Five weeks later an examination revealed a zone of dead and dried brown tissue extending in all directions from the point of inoculation. The dark spores of the fungus were present in this region. Beyond the dead tissue was a narrow, pinkish zone about 6 millimeters in width, and beyond that a tumeric-yellow band about 25 millimeters wide. The diseased tissue extended 7 to 10 centimeters from the focus of infection. It was brown to drab in color beyond the yellow zone and had no well-defined margin. It is difficult to determine the extent of diseased areas because the excised tissue darkens rapidly in the air.[6]

Roots of the date palm were likewise tested. On the northwest side of a seedling palm, 15 roots about 12 millimeters in diameter were carefully uncovered. Eight of these were inoculated; some by simply placing agar inoculum on the unbroken surface, and others by inserting the fungus in a 12-millimeter longitudinal slit made with a scalpel and covering the place of inoculation with moist cotton and waxed paper. All of the inoculated roots decayed, the affected portions being a soft, moist, brown decay which became a darker brown as the fungus fruited. In five weeks the affected tissue extended from 5 to 15 centimeters in both directions from the point of inoculation along the root. The inoculated roots, wounded and unwounded, showed no decay. The organism was reisolated from the diseased stem and roots.

VARIETAL SUSCEPTIBILITY

The midrib-scorch form of the disease has been found on all varieties of date palms growing in the Southwest, except the Tazizaoot. Although the fungus was first found causing inflorescence decay on the Deglet Noor, this variety in the Coachella Valley is perhaps one of the least susceptible to other forms of the disease. The Thoory variety appears to be very susceptible to the midrib-scorch form of the malady. The fibers of the midribs of the outer whorls of leaves seem to bind, and as growth proceeds from the center, to injure the young emerging fronds, thus affording an excellent opportunity for infection

[6] R. B. Streets, of the University of Arizona, at the 1930 Date Growers' Institute reported orally on a disease of the stems of neglected date palms in Arizona having symptoms similar to those on trunk tissue described here. The name of the organism was not mentioned at the time, but later it was identified as a species of Thielavia (*6*).

by the black-scorch fungus, even in the absence of moisture. Winds probably accentuate this type of mechanical injury. The cross cuts

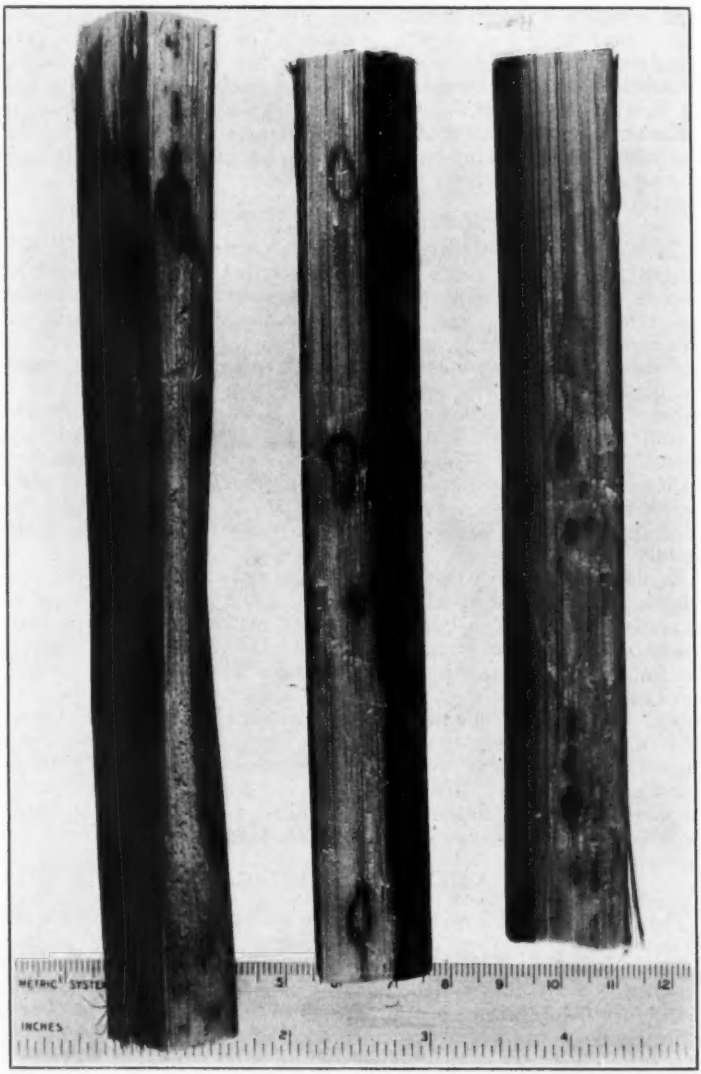


FIGURE 5.—Diseased internal tissues of petiole produced by artificial inoculation with the black-scorch organism

and V cuts mentioned earlier probably have the same origin. The abundance of the disease on the Thoory variety may be due to the greater incidence of this type of injury on that variety.

The Hayany, Amhat, Saidy, and Halawi varieties are likewise very susceptible, perhaps more so than the Deglet Noor. The disease was observed also on the Zaheedy, Menakher, Baklany, Guntar, Haloo, Fteemy, Besser Haloo, Nakleh-Zian, Sukar-Nabat, Horra, and and Koroch, but the number of individuals was too small to permit reliable comparison.

THE PATHOGENE

Patterson, Charles, and Veihmeyer (*10*) discuss the origin and synonymy of the fungus, stating that De Seynes found it on pineapple and described it under the name *Sporoschisma paradoxum*. Saccardo in 1892 gave it the genus name Chalara, the binomial becoming *Chalara paradoxa* (De Seynes) Sacc. The next year, Went in Java described a serious fungus disease of pineapple and designated the pathogene by the new generic name Thielaviopsis because it produced hyaline conidia endogenously in a manner similar to that of the genus *Thielavia* Zopf, and by the specific name *ethaceticus* because of the production of a pleasant, strong odor resembling ethyl acetate. Von Höhnel (*5*) observed that the fungus of De Seynes and that of Went were identical and established the priority of the specific name of the former, calling the fungus *Thielaviopsis paradoxa;* hence, *Thielaviopsis paradoxa* (De Seynes) Von Höhnel.[7] Butler (*2*) states that he found also a pycnidial stage of the fungus which might place it in the genus Sphaeronema. The pycnidia were globose, hairy, and ostiolate, the ostiole being at the tip of a long bristlelike neck. The pycnidiospores were small (10 to 12μ by 3μ), hyaline, unicellular. Patterson et al. (*10*) mention the appearance of pycnidia in their cultures of the fungus, but do not describe this stage. Thus far the writers have observed no pycnidial stage on specimens or on the various laboratory media.

The literature in general describes the fungus as having creeping, almost hyaline hyphae which bear two spore forms: Microconidia, which are small (10 to 15μ by 3.5 to 5μ), cylindrical, hyaline, and formed uniseriately within a hyphalike conidial case; and macroconidia (16 to 19μ by 10 to 12μ), which are extruded in chains from the tips of short lateral hyphae, and which are brown, thick-walled, and ovate. Grown on glucose-potato agar at 27° C., a culture 2 weeks old had conidia of the following dimensions: The so-called macroconidia with thick walls, length 11 to 17μ, width 7 to 15μ; brown conidia that were extruded from conidiophores of the same morphology as those that bear the hyaline so-called microconidia, length 6 to 23μ, width 4 to 8.5μ; and hyaline microconidia, length 5 to 15μ, width 3 to 7μ. The contents of both kinds of conidia vary greatly and may be very guttulate and granular to perfectly homogeneous. As shown in Plate 1, *a*, the typical conidiophores bearing the microconidia are much elongated and swollen at the base, while those bearing the macroconidia (pl. 1, *b*) are approximately half as long as the first and of uniform diameter. However, these distinctions as to spores and conidiophores are very artificial, as one finds all gradations in size, color, and shape between the extremes described, and all the conidia are probably produced endogenously, although some are pictured which appear to originate

[7] Dade (*2a*) has found a fungus of the genus Ceratostomella which he considers to be the perfect stage of *Thielaviopsis paradoxa;* the name of the organism would thus become *Ceratostomella paradoxa*.

acrogenously. In germinating on glucose-potato agar, the protoplast of the mature macroconidium bursts through a longitudinal slit and forms a globule of naked protoplasm which proceeds to grow into mycelium. (Pl. 1, *c*.) In water, the conidia germinate directly by sending out a germ tube. Apparently the brown conidia need a rest period before they will germinate. The hyaline conidium germinates readily without a rest period, sending out one (pl. 1, *d*), occasionally two, germ tubes from any place on its periphery. The hyphae are subhyaline with cross walls and show a strong tendency to anastomose and to form branches at right angles to the parent hypha. (Pl. 1, *e*.)

PATHOLOGICAL HISTOLOGY

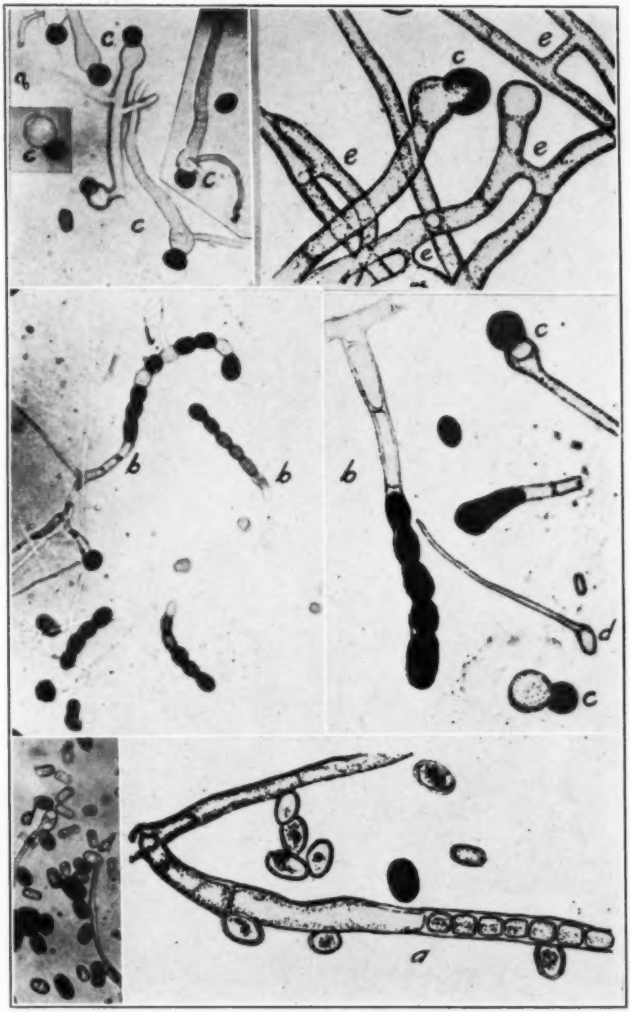
The cells of affected tissues turn brown as both walls and lumen become filled with gum. The formation of gum in palm fronds is not peculiar to this disease alone, for any wound induces a tendency to the formation of pentosanlike substances. In some sections the hyphae appear completely to fill some of the tracheae and parenchymatous cells. (Pl. 2.) These hyphae and the gum that forms in the pathological tissues may become so abundant as to interfere seriously with the transpiration stream and produce a permanent wilting of the pinnae several feet beyond the region invaded by the fungus. In addition to the intracellular growth, the fungus is found abundantly in the intercellular spaces but does not appear to grow in the region of the middle lamellae. Abundant fruiting occurs on the surface of a lesion and, as the decay progresses, within the disintegrated tissues.

CONTROL

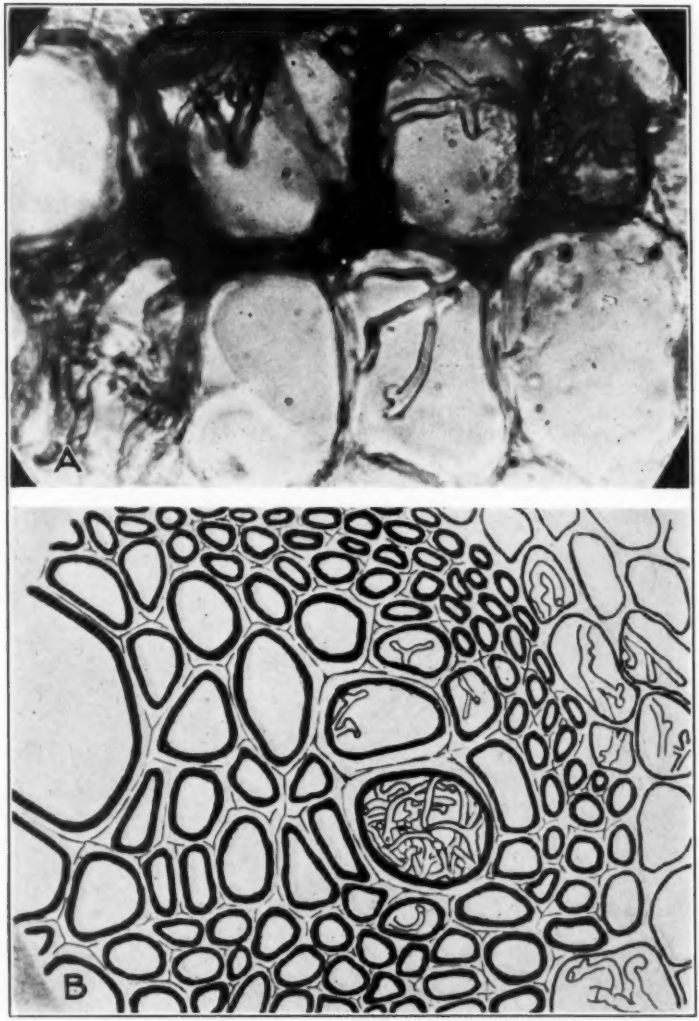
In the West Indies, dipping in 4–5–40 Burgundy or Bordeaux mixture gave adequate control of the disease on sugarcane sets. In Jamaica, where the malady occurs on coconuts, the diseased tissues are excised and the wounds dressed with a mixture of equal parts of copper sulphate, salt, and lime. Patterson et al. (*10*) found formaldehyde gas (1,200 cubic centimeters commercial formalin per 1,000 cubic feet) effective in controlling the fungus on pineapples, even when the organism was inserted to a depth of half an inch. Simmonds [8] in Australia has utilized both benzoic acid and boric acid effectively in controlling decay of pineapples. In this work the copper fungicides were less effective than the two organic acids.

In the case of date palms it seems advisable to prune out the affected fronds, leaf bases, and inflorescences, and to protect the pruning cuts and surrounding tissues with some disinfectant. Some preliminary laboratory experiments made by the writers indicate that copper sprays and dusts may be effective. Bordeaux dust, a 5–5–50 Bordeaux mixture, and ammoniacal copper carbonate inhibited germination of the conidia in a weak glucose-potato broth or in 10 per cent sucrose solution, the last-named fungicide being slightly less effective than the Bordeaux. Calcium monosulphide dust, dry lime sulphur, liquid lime sulphur, 1 per cent boric acid, 1 per cent benzoic acid, and 1 per cent formalin were likewise effective in inhibiting germination. All the chemicals in liquid form except

[8] Verbal communication.



Left, photomicrographs, × approximately 200; right, tracings of photomicrographs showing: *a*, Microconidiophore with endogenous hyaline conidia; *b*, macroconidiophores with fuscous macroconidia; *c*, macroconidium germinating; *d*, microconidium germinating; *e*, anastomosing hyphae, × approximately 400



A, Photomicrograph of hypha in parenchyma of petiole, × approximately 320; B, tracing from a photomicrograph of transverse section of petiole, showing hyphae in trachael tubes and in parenchyma, × approximately 160

the formalin were atomized onto glass slides and allowed to dry before the spore suspension was applied with an atomizer. Flowers of sulphur dust under the conditions of the experiment was entirely ineffective in preventing germination.

SUMMARY

A fungous disease of economic importance has been found on date palms in California, Arizona, and northern Africa. A preliminary survey indicates that all varieties of the date palm are probably susceptible. The disease has been found occurring naturally on all parts of the plant except the roots and stem, and these latter organs have by artificial inoculation been found to be readily susceptible.

Typical lesions are dark brown to black, hard, carbonaceous, and in mass give the petioles, midrib, fruit strands, and fruit stalks a scorched appearance, which suggests "black scorch" as the common name. Many of the fruit strands may be completely severed by the attack and the crop materially lessened. Wounding was shown to be unnecessary for infection of the root, fruit strands, petiole, and pinnae. The decay is most serious when it attacks the terminal bud, either killing the palm, or, when not fatal, producing the so-called "fool disease" effect, in which the injured terminal bud grows out laterally, setting the normal growth of the palm back several years.

Both the hyaline and the brown spores of the fungus *Thielaviopsis paradoxa* (De Seynes) Von Höhnel are found on the surface of the lesions. The conidia originate endogenously in uniseriate chains from subhyaline conidiophores. The optimum temperature for the fungus in culture lies between 24° and 27½° C.; it makes very little growth at 32°. The brown spores apparently need a rest period before germination. The hyaline conidium germinates readily without a rest period, sending out one, and occasionally two, germ tubes from any place on its periphery. In germinating on glucose-potato agar the protoplast of the mature macroconidium bursts through a longitudinal slit, liberating a globule of naked protoplasm which proceeds to grow into mycelium. The hyphae are subhyaline with cross walls and show a strong tendency to anastomose and to form branches at right angles to the parent hyphae.

A histological study of the petiole of a diseased frond showed the fungus growing intracellularly in tracheae and parenchyma and intercellularly in the intercellular spaces but not in the middle lamellae.

To control the malady, the affected fronds, leaf bases, and inflorescences should be pruned out and the pruning cuts and surrounding tissues protected with some disinfectant. Preliminary experiments indicate that copper sprays, dusts, and various other chemicals may be effective.

LITERATURE CITED

(1) Ashby, S. F.
1918. leaf bitten diseases of coconuts. Jour. Jamaica Agr. Soc. (1917) 21: 269–273.

(2) Butler, E. J.
1906. fungus diseases of sugar-cane in bengal. India Dept. Agr. Mem., Bot. Ser. 1 (3): 1–53, illus.

(2a) Dade, H. A.
1928. ceratostomaella paradoxa, the perfect stage of thielaviopsis paradoxa (de seyenes). Brit. Mycol. Soc. Trans. 13: 189–1924, illus.

(3) EDGERTON, C. W.
1910. SOME SUGAR CANE DISEASES. La. Agr. Expt. Sta. Bul. 120, 28 p., illus.
(4) FAWCETT, H. S.
1931. CULTURE AND DISEASES OF THE DATE PALM IN NORTH AFRICA. Date Growers Inst. Ann. Rpt. 8: 18–23.
(5) HÖHNEL, F. VON
1904. ZUR KENNTNIS EINIGER FADENPILZE. Hedwigia 43: 295–299.
(6) KILLIAN, C., and MAIRE, R.
1930. LE BAYOUD, MALADIE DU DATTIER. Bul. Soc. Hist. Nat. Afrique Nord 21: 89–101, illus.
(7) KLOTZ, L. J.
1931. INVESTIGATIONS ON DATE PALM DISEASES. Date Growers Inst. Ann. Rpt. 8: 14–18.
(8) ——— and RABY, E. C.
1930. A DISEASE OF DATE PALM INFLORESCENCES. (Abstract) Phytopathology 20: 852–853.
(9) ORIAN, G.
1928. LE "BITTEN-LEAF DISEASE" DU COCOTIER. Rev. Agr. Maurice 38: 86–87. Abstract in Rev. App. Mycol. 7: 629. 1928.
(10) PATTERSON, F. W., CHARLES, V. K., and VEIHMEYER, F. J.
1910. PINEAPPLE ROT CAUSED BY THIELAVIOPSIS PARADOXA. U. S. Dept. Agr., Bur. Plant Indus. Bul. 171: 15–38, illus.
(11) SUNDARARAMAN, S., KRISHNAN NAYAR, C., and RAMAKRISHNAN, T. S.
1928. THE STEM-BLEEDING DISEASE OF ARECANUT (ARECA CATECHU) CAUSED BY THIELAVIOPSIS PARADOXA VON HÖHN. Agr. Research Inst., Pusa, Bul. 169, 12 p., illus.

THE GLOSSY CHARACTER (gl_3) IN MAIZE AND ITS LINKAGE RELATIONS[1]

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INTRODUCTION

Glossiness in maize seedlings is inherited as a simple Mendelian recessive. At least three genetically different types of glossiness have been discovered, each dependent upon the homozygous recessive condition of a single factor. Glossy seedlings are characterized by a shiny, smooth, waxy appearance. A normal maize seedling has a slight silvery sheen on the surface which a glossy one lacks. When glossy seedlings are sprinkled, the water collects in drops on the surface of the leaves, while in normal seedlings the leaves shed the water completely. Classification of the two types in a segregating generation is therefore facilitated by sprinkling the leaves with water. Glossiness is apparent as soon as the leaf emerges from the soil, and may be observed until the plant is about 20 to 30 inches tall, when it begins to fade. By the time inflorescences are formed this character can no longer be differentiated.

The purpose of this study was to determine the normal mode of inheritance of glossiness (gl_3) in seedlings and its linkage relations, and also to investigate the physical properties of the character and its effect upon the vigor of plants possessing it.

REVIEW OF LITERATURE

During the last 20 years knowledge of linkage relationships in maize has developed to a point where the completeness of the chromosome map is second only to that of Drosophila. The comparative ease of culture, wide range of adaptation, type of inflorescence, large number of seeds per ear, and numerous observable genetic characters, all combine to make maize a good subject for genetic studies.

Beginning in 1929, Emerson and his coworkers at Cornell have each year summarized all the available linkage data in maize. The 1930 compendium lists approximately 100 genes combined into 10 linkage groups.[3] Populations for each cross are large, ranging from 500 to as many as 300,000 plants. Several 3-point relationships are given.

In 1921 Brunson discovered the strain now known as gl_1, in a first-year self progeny of a yellow dent corn from Illinois. A report of this discovery has never been published. In 1924 Kvakan (*8*)[4] reported a linkage relation of glossy seedling and brown aleurone. This was the first reference to the glossy character in the literature.

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[3] This material was as yet unpublished when the present paper was submitted.

[4] Reference is made by number (italic) to Literature Cited, p. 173.

The strain of glossy used was the simple Mendelian recessive discovered by Brunson, which has since been named gl_1. The linkage relations of glossy seedling (gl_1) were summarized by Brunson in 1926 (*2*).

Hayes and Brewbaker (*4*) studied glossy seedlings in maize and made crosses between various strains. They described the appearance of glossy seedlings and reported two new ones phenotypically indistinguishable but genotypically distinct from the one obtained by Brunson. The 3 strains were designated gl_1 (Brunson's), gl_2, and gl_3. Like gl_1, the new strains were each simple Mendelian recessives. It was stated that glossy seedlings appear rather frequently in selfed strains from various varieties. The results from crosses of 14 unknown glossies were given, each of these proving to be some one of the 3 reported.

In addition to establishing the existence of the two new glossy strains, Hayes and Brewbaker (*5*) found gl_2 to be linked with *Fl* (flinty endosperm). Finally, in 1930 Brewbaker and Hayes (*1*) published extensive data on the *B–lg* group, concluding that the order of the genes appeared to be (v_4–*Fl*–ts_1)?–*sk*–gl_2–*lg*.

Except for a brief description of the glossy character by Hayes and Brewbaker (*4*), no investigation of the anatomy of glossiness appears to have been reported.

APPLICATION OF ORGANIC SOLVENTS TO THE LEAF SURFACE AS A MEANS OF ASCERTAINING THE ANATOMICAL NATURE OF THE GLOSSY CHARACTER

As was stated earlier, sprinkling with water helps in differentiating the two types of seedlings. Water will collect in drops on a glossy leaf but is shed by the leaves of normal seedlings. This distinction may be due to a difference in interfacial tension between the two systems concerned, that is, water-glossy and water-normal interfaces. If a normal leaf is held in a level position a drop of water may be balanced upon it. However, the drop will be almost spherical and with the slightest tipping of the leaf will roll rapidly in one direction or the other. On a glossy leaf a drop of water of the same size will be flat and will generally adhere to the leaf even when the surface plane is in a vertical position. Sometimes the water will spread out to form a rather thin film over the leaf surface, though at ordinary room temperature it usually remains in drops.

If a normal leaf is sprinkled with ethyl alcohol the results are very similar to those produced by an application of water to a glossy leaf. Drops of alcohol about the same size and appearance as drops of water on glossy leaves form on the normal surface. At 20° C., or about room temperature, the surface tension of water is 72.8 dynes per cubic centimeter while that of ethyl alcohol under the same conditions is only 21.7 dynes per cubic centimeter. This fact indicates that the difference in the ability of the two seedling types to collect water drops is due to interfacial tension.

Further observations are compatible with those just mentioned. Alcohol applied to glossy leaves quickly spreads out into a thin film. Salt water or ice water, both of which are a little higher in surface tension than pure water at room temperature, are readily repelled by normal seedlings. What appears to be a similar increase in interfacial tension can be seen when ice or salt water is placed on glossy

leaves, though drops will still be formed. Since normal leaves are so completely free from water and the drops on glossy ones are fairly frequent, a bed of segregating plants sprayed with salt solution or ice water can be classified a little more readily than when the usual method is used.

Glossy seedlings are slightly more transparent than normal ones. This fact can best be observed by examining plants that are deficient in chlorophyll but segregating for glossiness. When a glossy and a normal leaf from white or very light colored virescent seedlings are held up to the light, the glossy leaf will appear more transparent. If the leaves are soaked in chloroform for 5 to 10 minutes this difference will disappear and the normal will transmit as much light as the glossy. Furthermore, if a normal green seedling is washed thoroughly with chloroform or immersed in it for about five minutes it will become almost indistinguishable from a glossy plant. After this treatment the normal seedlings will collect water drops just as do the true glossy ones. The same effect may be produced with ether, but not with alcohol. It should be mentioned that after the chloroform washing, although normal leaves will collect water readily, the shape of the drops suggests that the interfacial tension is slightly higher than on real glossy leaves. However, this point requires further investigation with more refined technic.

Figure 1 shows a glossy seedling and a normal seedling on which one leaf was treated with chloroform as described above.

It is significant that dried leaves may also be classified into normal and glossy by means of the water test. If the shape of the epidermal cells had any great influence on the glossy properties it would seem that drying the leaves should destroy the difference. Furthermore, the treatment of dried normal leaves with chloroform will make them appear like glossy ones, as in the case of fresh leaves.

COMPARISON OF YIELDS FROM GLOSSY AND NORMAL PLANTS

A measure of the possible reduction in vigor, if any, associated with the glossy condition was obtained in the following manner. A segregating generation of the corn was grown in rows 3 feet apart with single plants in each hill and the hills 1 foot apart. One hundred and twenty-seven pairs of glossy and normal plants growing side by side were selected, and when mature, the ears of each plant were harvested individually. Care was taken to avoid having vacant hills either between the normal and glossy or in the nearest space on either side of the pair. The corn was then dried to a uniform moisture basis and the weight taken. In 61 of the pairs the normal plant was the more productive; in the other 66 pairs the glossy plant was the more productive. The average yield of normal plants was 102.09 gm. and of the glossy plants 100.44, a difference of 1.65 ± 4.32 gm. In harmony with common observation, these results indicate that the presence of the glossy character detracts little, if any, from the general vigor of the plant. This vigor of homozygous glossy strains is one of the things that make the character particularly desirable for inheritance studies. These results indicate that the glossy character may be valuable as a marker in inbred lines or in crosses of corn used for commercial production, so that off pollinations may be readily detected and rogued out.

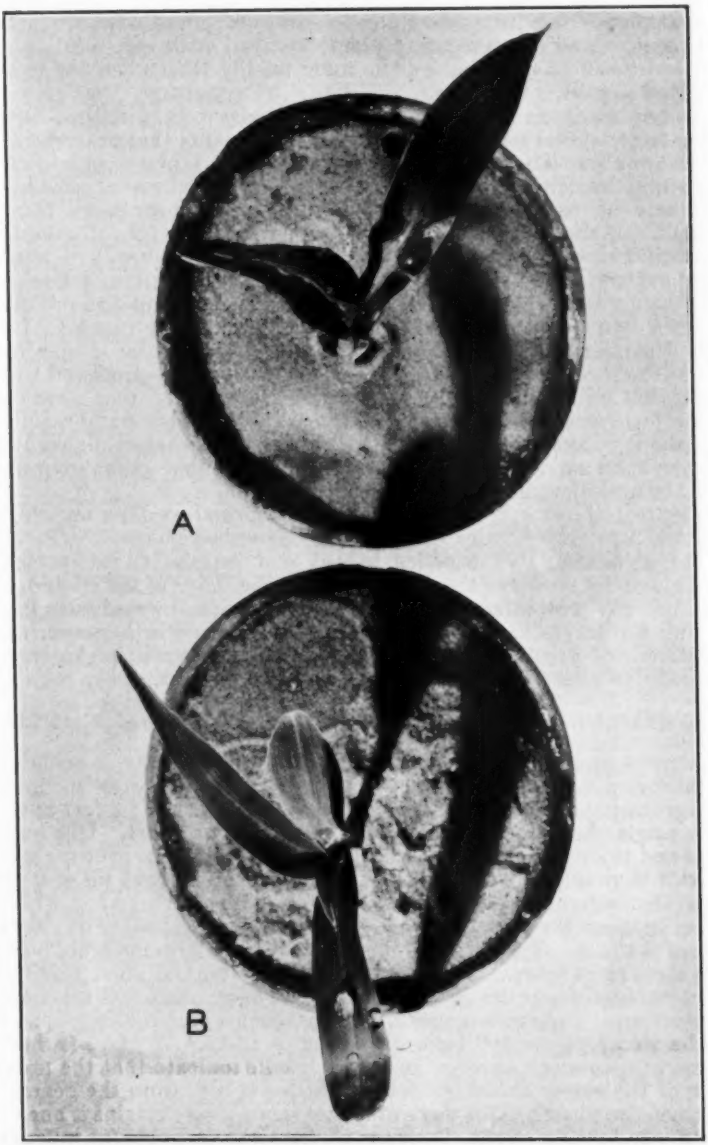

FIGURE 1.—A, Typical glossy maize seedling that has been sprinkled with water. Note the water adhering to the surface. B, Normal maize seedling. The lowermost leaf has been washed with chloroform and the whole plant subsequently sprinkled with water. Several drops of water may be seen on the one leaf that is a "manufactured" glossy

INHERITANCE STUDIES

METHODS

With a view to finding its linkage relation, a pure gl_3 culture was crossed with as many chromosome "markers" as were available. The work was started in 1927. The F_1 progenies from these crosses were back crossed to the double recessive in each case when it was practicable to do so. Some difficulty was experienced in getting double recessives. Where back crosses were not made the F_1 progenies were selfed to produce an F_2 population, and segregation of glossy in relation to the other character was studied by that means.

Since the glossy character may be differentiated in the seedling stage, a cross involving any other seedling difference or an endosperm character may be grown in the greenhouse and counts made there of segregating generations. This fact was taken advantage of in studying the crosses of gl_3 with *lg*, *sh*, *wx*, *fl*, and the four aleurone color factors *A*, *C*, *R*, and *Pr*.[5]

The recombination values from the back crosses were calculated by dividing the number of crossover gametes by the total number, since the constitution of the gametes produced by an F_1 progeny can be read directly in a back cross. In the case of the F_2 populations, recombination percentages and their probable errors were calculated from tables compiled by Immer (*6*), which are based on the work of Fisher and Balmukand (*3*).

TABLE 1.—*Linkage relations of gl_3 with the su-Tu group in maize*

Culture No.	Year	Gene	Linkage phase	Number of individuals [a]				Total population	Recombination percentage
				AB	Ab	aB	ab		
H76	1928	*su*	Repulsion F_2	2,078	653	924	43	3,698	25.1±0.48
H200×H210	1929	*su*	Repulsion, back cross	78	255	271	82	686	23.3±1.08
H260×H9	1930	*su*	Repulsion	148	576	614	169	1,507	26.6± .77
H26×H77	1928	*Tu*	do	1,093	172	107	1,195	2,567	10.88± .41

[a] *Aa* stands for $Gl_3\ gl_3$; *Bb* represents the other factor pair.

LINKAGE RELATIONS OF Gl_3 WITH THE *su-tu* GROUP

Table 1 gives the results of two back crosses and of one F_2 population which show that gl_3 is linked with *su*. The F_2 generation, consisting of 3,698 individuals, gave a recombination percentage of 25.1 ± 0.48. In one back cross, which numbered 686 plants, there was a recombination value of 23.3 ± 1.08 and in the other back cross, which contained 1,507 plants, there was a recombination value of 26.6 ± 0.77. The variations in these percentages (23.3, 25.1, and 26.6) do not appear significant in the light of their probable errors.

One back cross involving gl_3 and *Tu* was obtained. The result here was a recombination percentage of 10.88 ± 0.41.

According to the most recent summary of Emerson and his coworkers, data published by Jones and Gallastegui in 1919, and later and more extensive data from Eyster and from Emerson, give an average

[5] The genetic symbols used in the text are as follows: gl_1, glossy seedling; gl_2, glossy seedling; gl_3, glossy seedling; *sh*, shrunken endosperm; *wx*, waxy endosperm; *fl*, floury endosperm; *Fl*, flinty endosperm; *su* sugary endosperm; *Y*, yellow endosperm; *Tu*, tunicate ear; ts_1, tassel seed; *br*, brachytic; v_2, virescent seedling; *lg*, liguleless leaf; g_1, golden plant; *A*, *C*, *R*, and *Pr*, aleurone color factors; *B*, intensifier of plant color

recombination value of 28.6 per cent for *su* and *Tu*. Therefore the position of the genes is *su*–gl_3–*Tu*. The mean of the three crosses here reported involving *su* gave a distance of 25 units from *su* to gl_3. The distance from gl_3 to *Tu* (10.88) added to this gives a total of approximately 36, as compared to 28.6 for the distance from *su* to *Tu*. The distance, then, from *su* to *Tu* as previously reported is 7.4 units less than the sum of the distances *su* to gl_3 and gl_3 to *Tu* according to the data here presented. The probable error of the difference, 7.4 units, would be about 1 unit or slightly more since the probable errors of the linkage values concerned are all of approximately that size. In other words, this difference, though not very large, is statistically significant.

FIGURE 2.—Seedlings from the segregating back-cross generation of the cross H260×H9 made in 1930; photographed in the greenhouse

This is as it should be if the amount of double crossing over between two genes is directly proportional to their distance apart (*7*).

Figure 2 shows the seedlings from the segregating back-cross generation of the cross H260×H9 made in 1930. (Table 1.) This cross was made in the repulsion phase, that is, gl_3 gl_3 *Su Su* × Gl_3 Gl_3 *su su*. The seedlings shown in the lower right half of the illustration are from starchy seeds, while those in the upper half are from sugary ones. The seedlings were sprinkled with water just before the picture was taken and the glossy ones may be distinguished by the drops of water on them. It will be noted that there are many more glossy seedlings in the starchy group.

TABLE 2.—*Recombination values for Gl_3 gl_3 with factor pairs in known linkage groups in maize*

[Linkage phase, repulsion F_2]

Culture No.	Year	Gene	Linkage group	Number of individuals [a]				Total population	Recombination percentage
				AB	Ab	aB	ab		
H75	1928	*sh*	*C–wx*	2,703	818	810	304	4,635	53.0±0.71
H199	1929	*sh*	*C–wx*	2,572	711	801	238	4,322	51.0± .76
H73	1928	*wx*	*C–wx*	1,964	661	722	222	3,569	48.7± .86
H75	1928	*R*	*R–g_1*	2,580	941	808	306	4,635	50.5± .73
H72	1928	*R*	*R–g_1*	1,049	844	322	281	2,496	51.1±1.00
H76	1928	*R*	*R–g_1*	1,642	897	676	291	3,506	46.6± .88
H79	1928	*ts_1*	*B–lg*	1,199	361	314	79	1,953	47.5±1.17
H78	1928	*lg*	*B–lg*	677	264	265	80	1,286	46.4±1.46
H71	1928	*Fl*	*B–lg*	1,239	1,177	374	399	3,189	51.6± .88
H71	1928	*Y*	*Y–Pl*	1,793	623	597	176	3,189	47.7± .92
H82	1928	*br*	*P–br*	1,049	245	293	67	1,654	49.6±1.25
H72	1928	*Pr*	*Pr–v_2*	649	400	202	120	1,371	49.5±1.37
H76	1928	*Pr*	*Pr–v_2*	1,285	357	483	193	2,318	55.1± .99
H199	1929	*Pr*	*Pr–v_2*	2,420	863	770	269	4,322	49.7± .77

[a] *Aa* stands for Gl_3 gl_3; *Bb* stands for other factor pair concerned.

PROOF OF INDEPENDENCE OF gl_3 FROM SEVERAL LINKAGE GROUPS

In Table 2 data are presented from 16 F_2 populations, ranging from about 1,300 to 4,600 plants. Each of these populations gave a recombination percentage not deviating significantly from 50 per cent. The genes employed are *sh*, *wx*, *R*, *ts_1*, *lg*, *Fl*, *Y*, *br*, and *Pr*. These data indicate that gl_3 is not carried in chromosome groups *C–wx*, *R–g_1*, *B–lg*, *Y–Pl*, *P–br*, or *Pr–v_2*.

SUMMARY

The homozygous glossy condition (gl_3) has little, if any, effect on the general vigor of a corn plant. The true difference between normal and glossy seedlings appears to lie in the leaf cuticle. Exactly what this difference is has not been determined.

Inheritance studies have placed gl_3 in the *su–Tu* linkage group. The order of the genes is *su–gl_3–Tu*.

LITERATURE CITED

(1) BREWBAKER, H. E., and HAYES, H. K.
1930. GENETIC FACTOR RELATIONSHIPS IN THE B–LG GROUP IN MAIZE. Jour. Amer. Soc. Agron. 22: 1035–1040.

(2) BRUNSON, A. M.
1926. THE RELATION OF INHERITANCE STUDIES TO CORN IMPROVEMENT. Jour. Amer. Soc. Agron. 18: 308–314.

(3) FISHER, R. A., and BALMUKAND, B.
1928. THE ESTIMATION OF LINKAGE FROM THE OFFSPRING OF SELFED HETEROZYGOTES. Jour. Genetics 20: 79–92, illus.

(4) HAYES, H. K., and BREWBAKER, H. E.
1928. GLOSSY SEEDLINGS IN MAIZE. Amer. Nat. 62: 228–235.

(5) ——— and BREWBAKER, H. E.
1929. LINKAGE STUDIES OF FACTOR PAIRS FOR NORMAL VS. GLOSSY AND FLINTY VS. FLOURY ENDOSPERM IN MAIZE. Amer. Nat. 63: 229–238.

(6) IMMER, F. R.
1930. FORMULAE AND TABLES FOR CALCULATING LINKAGE INTENSITIES. Genetics 15: 81–98, illus.

(7) JENNINGS, H. S.
1923. THE NUMERICAL RELATIONS IN THE CROSSING OVER OF THE GENES, WITH A CRITICAL EXAMINATION OF THE THEORY THAT GENES ARE ARRANGED IN A LINEAR SERIES. Genetics 8: 393–457, illus.

(8) KVAKAN, P.
1924. THE INHERITANCE OF BROWN ALEURONE IN MAIZE. N. Y. Cornell Agr. Expt. Sta. Mem. 82, 22 p.

EFFECT OF THE HYDROGEN-ION CONCENTRATION OF THE SOIL ON THE GROWTH OF THE BEAN AND ITS SUSCEPTIBILITY TO DRY ROOT ROT[1]

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INTRODUCTION

A more thorough knowledge of the environmental factors that affect the common bean (*Phaseolus vulgaris* L.) infected with the root parasite *Fusarium martii phaseoli* Burk. should be of considerable economic value. In certain sections of western New York the root rot caused by this organism is enphytotic, but the severity of the disease varies greatly, not only in different years but in different fields during the same year. In one field the lower roots of infected plants may be entirely rotted, leaving the plants supplied only by the surface roots; while in a neighboring field only the cortex of the tap-root and the finer rootlets will be invaded. In the latter case the reduction in yield is scarcely noticeable.

Certain environmental factors have been studied which might alter the severity of the disease and thus explain conditions such as those just described. It is the belief of Reddick[2] and of the writer[3] that while the soil temperatures found in the bean-growing sections of New York State may greatly influence the growth of the bean plant, they do not materially affect the course of the root-rot disease. Soil moisture, on the other hand, although a factor of some importance, has been found by the writer[4] to act only in a mechanical manner. If there is sufficient moisture in the soil, the roots, even though severely rotted, are able to supply water and inorganic food to the plant in such quantities that the reduction in yield of seed is slight. If the soil is dry, however, the yield may be reduced as much as 50 per cent. A third factor studied by the writer[5] directly concerns the pathogene. When *Fusarium martii phaseoli* is grown in pure culture its virulence is considerably reduced although such an attenuated culture may be restored to normal by growing it for several months on its host, the bean plant. It seems probable, although it has never been demonstrated, that a similar condition takes place in nature. After the fungus has grown saprophytically in the soil for a number of years it may cause only a light infection of the bean roots, but continued association with its host may build up its virulence.

The factors of soil moisture and the attenuation of the pathogene do not appear, however, to explain adequately all variations in the disease which are observed in the field. Other environmental factors

[1] Received for publication June 17, 1931; issued March, 1932.

[2] REDDICK, D. EFFECT OF SOIL TEMPERATURE ON THE GROWTH OF BEAN PLANTS AND ON THEIR SUSCEPTIBILITY TO A ROOT PARASITE. Amer. Jour. Bot. 4: 513–519. 1917.

[3] BURKHOLDER, W. H. THE EFFECT OF TWO SOIL TEMPERATURES ON THE YIELD AND WATER RELATIONS OF HEALTHY AND DISEASED BEAN PLANTS. Ecology 1: 113–123, illus. 1920.

[4] ——— THE EFFECT OF VARYING SOIL MOISTURES ON HEALTHY BEAN PLANTS AND ON THOSE INFECTED BY A ROOT PARASITE. Ecology 5: 179–187. 1924.

[5] ——— VARIATIONS IN A MEMBER OF THE GENUS FUSARIUM GROWN IN CULTURE FOR A PERIOD OF FIVE YEARS. Amer. Jour. Bot. 12: 245–253. 1925.

that might affect its development were therefore sought. The hydrogen-ion concentration of the soil is known to alter the course of certain plant diseases, and a few preliminary observations indicated that it might affect the development of the dry root rot of the bean. Accordingly, an investigation was undertaken to determine the influence of this factor on (1) the growth of the plant and (2) on its susceptibility to attack by the dry root-rot organism.

It has been the general feeling among growers that an alkaline soil is necessary for a good production of beans, and this idea has crept into certain textbooks on vegetable gardening and field crops. Scattered references to beans in the reports of the extensive, but by no means recent, experiments conducted at the Rhode Island Agricultural Experiment Station on the effect of soil acidity and liming on plant growth indicate that the bean readily tolerates acid soils.

The present investigation was divided into two parts: (1) Experiments conducted in the greenhouse, in which many of the external factors were controlled, and (2) field surveys in the bean-growing section of New York State.

GREENHOUSE EXPERIMENTS

Two series of controlled experiments were conducted, one during the early months of 1927 and the other in 1928. The experiments followed in outline earlier tests conducted by the writer [6] on the relation of soil moisture to this disease. Glazed jars of 1-gallon capacity filled with a rich garden loam of known hydrogen-ion concentration and water-holding capacity were used. The pH of the soil at the beginning of both the experiments was approximately 5.10. The hydrogen-ion concentration was determined electrometrically, a quinhydrone electrode being used. The water-holding capacity of the soil was determined by the Hilgard 1-cm. cup method.

In the first set of experiments, 50 culture vessels divided into 10 lots of 5 each were used. One lot was left untreated, and the hydrogen-ion concentration of each of the remaining lots was adjusted by the addition of sulphuric acid or sodium hydroxide. In 4 lots the hydrogen-ion concentration was increased by the addition of sulphuric acid. To each vessel in lot 1 was added 30 c. c. of a normal solution of sulphuric acid, and to each jar in lots 2, 3, and 4 were added, respectively, 70, 125, and 210 c. c. In 5 lots of vessels the hydrogen-ion concentration of the soil was lowered by the addition of a normal solution of sodium hydroxide in the following quantities: To each vessel in lot 1, 26.25 c. c.; lot 2, 52 c. c.; lot 3, 87.5 c. c.; lot 4, 122.5 c. c.; and lot 5, 175 c. c. The treated jars were allowed to stand until the soil became friable, and the soil was then mixed thoroughly and softened. This was necessary in the case of jars receiving large amounts of sodium hydroxide because the surface soil in these vessels became baked on drying.

The seed used in the experiment was a pure line of Wells Red Kidney. At the time of planting (March 13) a culture of the bean nodule organism was introduced into each vessel. A water suspension of spores of a recently isolated strain of *Fusarium martii phaseoli* was poured about the seed in three vessels in each lot of five, and an equal quantity of sterilized water was added to the check vessels. The soil

[6] BURKHOLDER, W. H. Op. cit. (See footnote 4.)

moisture throughout the entire series was held as uniform as possible by the method used in previous experiments.[7] At the beginning of the experiment the moisture content was adjusted to 50 per cent of the water-holding capacity of the soil. When the seed germinated all seedlings but two were removed, and the soil moisture was lowered to 45 per cent of its water-holding capacity. This percentage was maintained until blossoming time, when it was further lowered to 35.

The data obtained in this set of experiments are shown in Table 1. Three check cultures had to be discarded because of chance infection. The cultures are arranged in the table according to the amount of acid or alkali that each group received, and this arrangement follows, more or less, the hydrogen-ion concentration of the soil. The pH determinations were made at planting and at harvest. The hydrogen-ion concentration, it will be noted, decreased steadily in all vessels except four, and the change in a few others was slight. This decrease was on both untreated soil and on soil treated with acid and with sodium hydroxide.

TABLE 1.—*The effect of various hydrogen-ion concentrations of the soil, determined at planting and at harvesting, on the yield of healthy bean plants and on those infected with Fusarium martii phaseoli*

Experiment No.*	pH of the soil		Yield	Experiment No.*	pH of the soil		Yield
	At planting	At harvesting			At planting	At harvesting	
			Grams				*Grams*
1C	6.71	7.27	5.18	25C	5.13	5.94	8.34
2C	6.75	7.19	4.48	26I	5.13	5.36	5.79
3I	6.67	6.75	2.22	27I	5.07	5.69	5.18
4I	6.73	6.75	.91	28I	5.16	5.69	7.45
5I	6.69	6.85	1.69	29C	4.86	5.39	5.34
6C	6.34	6.75	5.12	30I	4.85	5.34	6.02
7I	6.30	6.68	2.97	31I	4.83	5.18	4.98
8I	6.34	6.72	6.00	32I	4.88	5.31	4.40
9I	6.28	6.70	4.97	33C	4.66	4.77	4.54
10C	5.79	6.32	5.46	34C	4.62	5.00	3.70
11C	5.92	6.73	5.87	35I	4.60	4.67	3.00
12I	5.78	6.36	5.24	36I	4.63	4.88	4.31
13I	6.07	6.71	5.26	37I	4.66	4.89	2.20
14I	5.95	6.53	6.24	38C	4.43	4.57	1.49
15C	5.62	6.56	8.72	39C	4.44	4.44	1.65
16I	5.66	6.05	4.54	40I	4.39	4.72	1.05
17I	5.54	6.16	5.91	41I	4.31	4.47	.61
18I	5.63	6.14	5.09	42I	4.42	4.76	1.07
19C	5.43	6.30	7.28	43C	4.16	4.10	------
20C	5.50	6.08	6.85	44C	4.09	------	.09
21I	5.49	5.40	5.37	45I	4.09	4.25	------
22I	5.41	5.66	5.30	46I	4.11	4.04	------
23I	5.47	5.93	6.16	47I	4.09	4.19	.33
24C	5.12	5.60	7.53				

* When experiment numbers are followed by C, they refer to checks; when by I, they refer to inoculated plants.

A possible objection to this set of experiments is that the hydrogen-ion concentration of the soil does not extend to the alkaline side of neutrality. Preliminary tests on adjusting small quantities of this soil with sodium hydroxide showed that it should reach a pH of 8. However, because of the large quantities of soil used and the method of handling, it was not possible to obtain pH values above 7. On the other hand, when the soil was adjusted with sulphuric acid the

[7] BURKHOLDER, W. H. Op. cit. (See footnote 4.)

hydrogen-ion concentrations determined in the preliminary tests were reached.

A second experiment was conducted in the early months of 1928. This experiment differed from the first in that an acid soil was used and the hydrogen-ion concentration was adjusted with hydrated lime. Forty-five culture vessels were divided into nine lots of five each. Lot 1 remained untreated, but to each jar in lot 2, 4 gm. of lime was added and to each jar in the remaining lots the application was successively increased by 4 gm. The lime was thoroughly mixed with the soil and allowed to stand for eight days, the moisture content being held at 50 per cent of the water-holding capacity. At the end of the eighth day lime was still visible in the jars that received large applications. In order that an equilibrium might be hastened all the jars were steam sterilized. The result of this process was not entirely satisfactory, however, for the hydrogen-ion concentration of the soil in all the jars decreased appreciably, whether or not they had received an application of lime. Nevertheless, the experiment was continued since the series covered the alkaline side of neutrality, the side that had not been reached in the previous experiments. Except for the use of lime and the sterilization of the soil, these jars were handled like those in the first experiment. The data collected are presented in Table 2. Where some of the check cultures are missing it is due to the fact that the plants became infected and were discarded.

TABLE 2.—*The effect of various hydrogen-ion concentrations of the soil at time of planting, podding, and harvesting, on the yield of healthy bean plants and on those infected with Fusarium martii phaseoli*

Experiment No.[a]	pH of the soil			Yield	Experiment No.[a]	pH of the soil			Yield
	At planting	At podding	At harvesting			At planting	At podding	At harvesting	
				Grams					*Grams*
1C	8.43	7.76	8.27	1.57	22I	7.76	6.96	4.49	
2C	8.04	7.69	7.82	4.03	23I	7.66	7.34	7.74	6.12
3I	8.11	7.69	7.66		24C	7.51	7.39	7.44	6.10
4I	7.72	7.72	7.79	.28	25I	7.40	7.08	7.27	4.74
5I	7.89	7.56	7.79	.95	26I	7.27	6.98	7.05	3.87
6C	8.09	7.69	8.23	4.72	27I	7.30	7.35	7.52	7.92
7C	8.03	7.77	8.11	4.90	28C	7.15	7.42	7.17	8.52
8I	8.01	7.79	8.25	4.85	29C	7.24	7.07	7.08	8.17
9I	8.03	7.79	8.28	4.37	30I	7.39	7.32	7.32	6.92
10I	7.84	7.29	7.61	.20	31I	6.73		6.61	5.51
11C	7.83	7.62	8.01	7.05	32I	7.57	7.47	7.37	3.27
12I	7.86	7.59	8.30	6.15	33C	6.91	6.90	6.44	7.72
13I	7.91	7.44	7.97	2.90	34I	6.93	6.10	6.31	4.82
14I	7.89	7.89	7.86	5.97	35I	7.24	7.42	7.07	4.92
15C	7.47	6.71	7.66	5.70	36I	6.90	6.86	6.95	1.88
16C	8.21	8.08	8.35	7.55	37C	6.41	6.48	6.61	1.75
17I	7.78	7.07	7.59	5.15	38C	6.41	6.02	5.90	1.32
18I	7.90	7.49	7.94	3.01	39I	5.87	6.30	5.87	3.05
19I	7.92	7.52	7.74	4.75	40I	6.00	5.98	5.51	2.77
20C	8.08	7.89	8.05	7.67	41I	6.34	6.50	6.05	5.27
21I	7.44	7.30	7.54	6.98					

[a] When experiment numbers are followed by C, they refer to checks; when by I, they refer to inoculated plants.

The data in Table 2 are grouped in a manner similar to those in Table 1; that is, according to the amount of lime that each jar received. This arrangement follows, only in a general way, the hydrogen-ion concentration of the soil. The soil reaction of jars under like treatment varied considerably for no apparent reason, and the

variation from planting time to harvest was not always consistent. While a uniform series of cultures would have been highly desirable it was not absolutely necessary, since the object of the experiment was to determine whether or not the hydrogen-ion concentration of the soil at any degree would have an inhibiting effect upon the disease.

In Tables 1 and 2 an attempt was made to use the yield of seed in each culture vessel as an index of the amount and severity of the root rot present. This attempt, however, was not successful. There appeared to be too many factors besides root rot that influenced the yield. Some of these were unknown and others were difficult to control. For example, in the second set of experiments the greenhouse in which the plants were growing was very warm for several days at blossoming time because of the brightness of the sun, and many blossoms dropped. The drop was uneven, and at harvest time it was noted that certain plants, in the check cultures expecially, had endeavored to even up this inconsistency by the production of late blossoms. The pods from these blossoms did not produce seed, however, so no record of them appears in the data.

In spite of the variations in yield shown in Tables 1 and 2 it may be seen that there is a decided tendency for plants from inoculated seed to produce fewer seed than the check plants. Moreover, this decrease in yield of plants from inoculated seed does not vary in any direct relationship with the hydrogen-ion concentration, and this at least would indicate that soil reaction has no effect upon the root rot of the bean. Furthermore, disregarding the data on yields, the same conclusions were reached when the roots of the plants were examined at harvest time. All roots of inoculated plants appeared to be equally infected, scarcely any variation in the severity of infection being discernible. This last observation is of greater importance than the data on yield, and mainly because of this finding further experiments of this type were discontinued.

Before leaving these experiments, however, it should be pointed out that the data in Table 1 seem to indicate that the red kidney bean will grow well in a soil with a fairly high hydrogen-ion concentration. A decrease in yield of healthy plants occurred only after the pH of the soil had dropped below 5.

FIELD SURVEYS

Along with the two sets of greenhouse experiments a survey of bean fields in western New York was made during August in 1928 and 1929. The surveys were made each year at as nearly the same time as possible so that the data would be comparable if a seasonal variation in the hydrogen-ion concentration of the soil occurred. On these surveys the following procedure was employed: First, the variety of beans was determined and an estimate was made of the yield, then the degree of severity of root-rot infection was noted. If other diseases or factors were present that might contribute to a decrease in yield they, too, were recorded. A group of bean plants was then selected which appeared typical of the field at large and three samples of soil were taken about the bean roots within a foot or more of each other. While the hydrogen-ion concentration might vary over the field, it was felt that a determination should be made near a typical group of plants. The three soil samples were mixed, placed in a paper bag, and taken to the laboratory for pH determinations.

In 1928 the survey began in southern Monroe County and proceeded south through Genesse County into the white marrow section of Wyoming County. The high alkaline soils were found in the limestone area near Garbut and the acid soils about Perry and Castile. The data from this survey are shown in Table 3. In 1929 the survey was made in some of the newer bean-growing sections, whereas in 1928 the survey was made in a section where beans had been grown for many years. This probably accounts for the fact that more fields were found to be contaminated with *Fusarium martii phaseoli* the first year than were found the second year. The data collected in 1929 are presented in Table 4.

TABLE 3.—*Hydrogen-ion concentration of the soil of bean fields contaminated with or free from the root parasite Fusarium martii phaseoli*

[Determinations made August, 1928]

Sample No.	pH of soil	Variety grown	Extent of infection	Yield of beans
1	8.67	Pea	Severe	Poor.
2	8.64	do	None	Excellent.
3	8.45	do	Severe	Fair.
4	8.30	do	Light	Good.
5	7.96	Medium	do	Fair.
6	7.75	Pea	Trace	Poor.[a]
7	7.66	do	None	Good.
8	7.32	Red kidney	Light	Fair.
9	7.03	Marrow	do	Good.
10	6.93	Blue pod medium	Moderate	Fair.
11	6.88	White marrow	Severe	(?)
12	6.83	do	Moderate	Fair.
13	6.64	Red kidney	Trace	Good.
14	6.58	White marrow	Very severe	Poor.
15	6.31	do	do	Do.
16	6.25	Red kidney	Light	Do.[b]
17	6.05	White marrow	Moderate	(?)
18	6.02	Red kidney	Light	Good.
19	5.95	White marrow	do	Do.
20	5.78	do	do	Fair.
21	5.54	do	Very severe	Poor.
22	5.32	do	Trace	Good.
23	5.29	do	Moderate	Do.
24	5.27	do	Severe	Fair.
25	5.27	do	Very severe	Poor.
26	5.19	do	do	Do.
27	5.02	do	Trace	Do.
28	5.02	Yellow eye	Light	Good.
29	5.00	White marrow	Moderate	Poor.
30	4.99	do	Light	Good.
31	4.99	do	Trace	Fair.
32	4.97	do	None	Good.
33	4.95	Yellow eye	Trace	Fair.

[a] Anthracnose also present in the field which might have decreased yield.
[b] Anthracnose and bacterial blight also present and might have decreased yield.

TABLE 4.—*Hydrogen-ion concentration of the soil of bean fields contaminated with or free from the root parasite Fusarium martii phaseoli*

[Determinations made August, 1929]

Sample No.	pH of soil	Variety grown	Extent of infection	Yield of beans
1	8.30	Pea	Very severe	None.
2	8.19	do	Trace	Poor.[a]
3	8.19	White marrow	Light	Good.
4	8.18	Red kidney	Trace	Fair.
5	8.08	White marrow	Severe	Poor.
6	7.97	do	do	Do.
7	7.84	Red kidney	None	Good.
8	7.84	Pea	Trace	Do.
9	7.79	Red kidney	None	Do.
10	7.57	White marrow	Moderate	Fair.
11	7.49	Red kidney	Trace	Good.
12	7.47	Blue pods	do	Fair.
13	7.40	White marrow	None	Good.
14	7.35	Red kidney	Trace	Fair.[b]
15	7.34	White marrow	Severe	Poor.
16	7.32	Red kidney	Trace	Good.
17	7.29	do	None	Fair.[c]
18	7.25	do	Light	Good.
19	7.25	White marrow	do	Do.
20	7.08	do	Very severe	Poor.[d]
21	7.07	do	Trace	Do.[a]
22	7.03	Pea	Severe	Do.
23	7.03	White marrow	Trace	Good.
24	6.98	Red kidney	Light	Do.
25	6.91	White marrow	Moderate	Fair.
26	6.91	Pea	None	Poor.[a]
27	6.71	White marrow	Moderate	Good.
28	6.70	do	None	Poor.[b]
29	6.51	White imperial	do	Good.
30	6.44	Red kidney	do	Fair.[a]
31	6.39	do	do	Do.
32	6.27	Pea	do	Good.
33	6.02	White marrow	Moderate	Poor.[c]
34	5.97	Pea	None	Fair.
35	5.90	Red kidney	do	Good.
36	5.80	Pea	do	Do.
37	5.70	do	do	Fair.
38	5.68	do	Light	Do.[c]
39	5.62	Red kidney	do	Do.
40	5.54	White kidney	Trace	Do.
41	5.48	Pea	Severe	Poor.

[a] Dry weather may have decreased yield.
[b] Planted late, which may have decreased yield.
[c] Bacterial blight may have decreased yield.
[d] Insects may have decreased yield.

Tables 3 and 4 show that severe infection may be produced by the root-rot organism in a soil of fairly high hydrogen-ion concentration (pH 5.0) and in an alkaline soil (pH 8.0) as well. This is in harmony with the data gathered in the greenhouse experiments. The tables show further that the range of hydrogen-ion concentration of the soil in the bean-growing section of New York is not a limiting factor in crop production. A good yield may be produced in both acid and alkaline soils, and this also agrees with the data in Tables 1 and 2. If certain varieties of beans are less acid tolerant than others it is not apparent from these surveys.

CONCLUSIONS

The bean plant (*Phaseolus vulgaris*) appears to be little affected by the hydrogen-ion concentration of the soil, and thrives well in an acid or in an alkaline soil.

In the bean-growing section of New York State the susceptibility of the bean plant to *Fusarium martii phaseoli* is not affected by the hydrogen-ion concentration of the soil.

RELATION OF TEMPERATURE TO ANTHESIS AND BLOSSOM DROP OF THE TOMATO, TOGETHER WITH A HISTOLOGICAL STUDY OF THE PISTILS [1]

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INTRODUCTION

Under certain conditions and in practically all parts of the country large numbers of tomato blossoms fail to set fruit. This abortion and dropping of flowers may be so great as to reduce the crop materially. Abscission, which results in the dropping of the flowers, may occur before, during, or shortly after anthesis. This problem has received attention from investigators throughout the country. Usually, however, it has been considered from the standpoint of the nutrition of the plant and the influence of unfavorable weather conditions. It is not known whether these conditions affect the formation of the embryo sac, the fertilization process, or the development of the embryo and endosperm. This paper presents (1) the results of observations on the effect of temperature on anthesis and blossom drop and (2) the results of a histologic study of normally developing and dropping blossoms, with special reference to the development of the embryo sac, embryo, and endosperm.

REVIEW OF LITERATURE

Thompson [4] has enumerated the following as possible causes of blossom drop: (1) A sudden appearance of cold or cool weather when the plants are in blossom; (2) hard rains which may wash away the pollen or otherwise affect pollination; (3) very hot, dry weather, especially drying winds; (4) injury by thrips; and (5) rapid vine growth resulting from an excess of nitrogen in the soil.

Investigations to determine the causes of the shedding of tomato blossoms have been carried on in Oklahoma for at least 14 years by Morris, Booth, Herron, Cross, and White.[5] These investigators have studied the following as probable causes: (1) Extremely hot weather, (2) overrapid growth due to an excess of nitrogen fertilizers, (3) lack of proper nutrients in the soil, (4) deleterious substances in the soil, (5) insect injury, (6) hot dry winds, (7) lack of moisture, (8) extremes of temperature, (9) varietal susceptibility, (10) poor pollination, and (11) low humidity. Morris conducted variety, pruning, and soil-fertility tests but obtained successful results of only minor importance. He also shaded some of the plants but apparently found no direct evidence of the cause of the trouble. Booth continued the varietal studies, and also inaugurated studies in the relation of humidity, irrigation, diseases, thrips, and pollination to fruit produc-

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[2] The author is indebted to Ephraim Hixson and John Faulkner for assistance in a part of this investigation.

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[4] THOMPSON, H. C. VEGETABLE CROPS. 478 pp., illus. New York. 1923.

[5] MORRIS, O. M., BOOTH, N. O., HERRON, L. C., CROSS, F. B., and WHITE, R. E. FAILURE OF TOMATO BLOSSOMS TO SET FRUIT. Okla. Agr. Expt. Sta. unpublished data.

tion. The number of fruits produced under conditions resulting from irrigation was increased 18 per cent. Herron concluded from the results of windbreak tests that hot dry winds have little effect on the dropping of the blossoms. Blossom drop was not reduced by shading the plants nor by pruning and training them. Heavy fertilization with sodium nitrate, potassium sulphate, and acidulated rock phosphate applied in separate plots likewise failed to reduce the amount of blossom drop. Thrips, *Euthrips tritici projectus*, were found in abundance, but no constant relationship was observed between the number of thrips present and the number of blossoms that dropped.

Radspinner [6] continued the work at Oklahoma for several years, and in 1922 summarized the data. He noticed that the dropping was most severe when soil moisture was deficient or when the temperature was high or the humidity low. Fertility of soil influenced but slightly the dropping of blossoms.

Cross, working at Oklahoma in 1924 and 1925, divided the fruiting season into three periods. He found that blossoms were shed at all times during the season, but that the percentage shed was low during the first and third periods and high during the second. By far the greater number of blossoms produced during July and August were shed. Cross also found that a large percentage of shedding blossoms had injured floral parts. This he attributed to the fact that hot dry winds withdraw moisture from the organs, causing them to blacken and die. This injury prevents normal pollination and fertilization. He concluded that the greater part of the blossom drop was caused by excessively hot dry winds and intense heat of the sun.

Kendall [7] thinks that physiological conditions within the plant, as influenced by soil moisture and fertility, are the chief causes of floral abscission in the field. Kraybill [8] showed that the number of blossoms that dropped was influenced by the amount of available mineral nutrients under conditions of uniformity with respect to other external factors.

MATERIAL AND METHODS

All the tests were made at the Oklahoma Agricultural Experiment Station. The Bonny Best variety of tomato, grown during 1929 as a fall crop in the greenhouse and handled in a commercial manner, was used in these studies. All plants were pruned to one stem. Collections for the study of the development within the pistils after anthesis were made at certain intervals after emasculated blossoms had been hand-pollinated. Pollinations were made on the day of anthesis. Samples of naturally aborting flowers and emasculated, unpollinated flowers were collected at the same time that collections of developing flowers were made. The pistils were killed and fixed in Karpechenko's solution, dehydrated, embedded in paraffin, sectioned, and stained in the usual manner. Haidenhain's haematoxylin stain was used. For tracing pollen-tube growth the sections were also stained with resorcin blue after the second iron-alum solution in the schedule for the haematoxylin stain.

[6] RADSPINNER, W. A. EFFECTS OF CERTAIN PHYSIOLOGICAL FACTORS ON BLOSSOM DROP AND YIELD OF TOMATOES. Amer. Soc. Hort. Sci. Proc. (1922) 19: 71–75. 1923.

[7] KENDALL, J. N. ABSCISSION OF FLOWERS AND FRUITS IN THE SOLANACEÆ, WITH SPECIAL REFERENCE TO NICOTIANA. Calif. Univ. Pubs., Bot. 5: [347]–428, illus. 1918. (Thesis, Ph. D., Univ. Calif., 1917.)

[8] KRAYBILL, H. R. EFFECT OF NUTRITION ON THE NUMBER OF BLOSSOMS PER CLUSTER AND THE DROPPING OF BLOSSOMS IN THE TOMATO. Amer. Soc. Hort. Sci. Proc. (1925) 22: 371–374. 1926.

During the spring and summer of 1930 information was obtained as to the time and amount of anthesis and the time and amount of abortion of these flowers on plants grown out of doors. Ten plants of equal size and age were selected for study. The number of flowers opening each day on the different plants from June 8 to July 17 and the number dropping each day from June 12 to July 16 were recorded.

TABLE 1.—*Tomato anthesis from June 8 to July 17, 1930*

Date	Number of flowers opening on plant										Total	Average
	1	2	3	4	5	6	7	8	9	10		
June 8	1	0	1	0	0	0	0	0	0	0	2	0.2
9	1	2	0	1	0	0	0	0	0	0	4	.4
10	1	2	0	1	0	1	0	0	0	0	5	.5
11	3	2	4	1	1	0	2	0	2	0	15	1.5
12	1	1	0	0	0	1	0	1	0	0	4	.4
13	1	0	0	0	0	0	0	1	0	0	2	.2
14	0	1	0	0	1	0	0	0	0	1	3	.3
15	1	0	0	2	2	0	1	0	1	0	7	.7
16	0	1	2	1	0	2	0	2	0	1	9	.9
17	3	3	1	2	2	2	1	0	1	0	15	1.5
18	1	4	2	1	3	1	3	1	2	1	19	1.9
19	2	6	3	6	3	5	2	6	0	5	38	3.8
20	2	4	4	2	2	2	4	2	3	3	28	2.8
21	3	2	3	2	3	1	2	3	2	0	21	2.1
22	3	2	2	3	1	3	2	2	3	0	21	2.1
23	1	2	1	1	2	1	1	2	0	0	11	1.1
24	2	4	3	4	6	2	2	6	0	2	31	3.1
25	3	6	1	5	3	2	2	2	2	5	31	3.1
26	1	2	2	2	3	7	5	7	5	0	34	3.4
27	1	6	2	6	1	7	1	4	1	1	30	3.0
28	1	3	2	13	2	2	6	5	3	7	44	4.4
29	3	3	2	5	2	3	2	5	3	3	31	3.1
30	0	1	1	0	2	0	1	3	1	0	9	.9
July 1	1	1	1	0	2	3	2	3	2	4	19	1.9
2	1	4	0	0	4	1	5	5	4	4	28	2.8
3	5	0	4	1	3	1	5	1	2	3	25	2.5
4	3	2	2	0	3	2	4	3	1	0	20	2.0
5	1	1	1	3	6	3	6	2	7	2	32	3.2
6	2	2	1	0	5	0	6	2	6	3	27	2.7
7	3	0	2	1	0	1	2	2	0	2	13	1.3
8	1	1	1	3	3	3	5	2	2	2	23	2.3
9	0	0	4	2	2	4	4	2	1	1	20	2.0
10	1	0	0	2	0	2	1	1	1	3	11	1.1
11	3	0	0	3	2	6	3	2	1	3	23	2.3
12	2	1	2	4	3	3	5	0	3	5	28	2.8
13	5	0	6	3	3	3	6	0	2	4	32	3.2
14	2	0	3	2	5	3	7	0	4	5	31	3.1
15	2	1	1	0	2	2	5	3	0	1	17	1.7
16	2	1	4	4	1	1	2	1	0	0	16	1.6
17	4	2	2	4	1	2	3	1	0	1	20	2.0
Total	73	73	70	90	84	82	108	82	65	72	799	

OBSERVATIONS

INFLUENCE OF TEMPERATURE ON ANTHESIS AND BLOSSOM DROP

Table 1 gives the number of flowers that opened each day on the different plants from June 8 to July 17. After July 17 the taking of records was discontinued. The data show that tomato plants have no one definite flowering peak. In most plants there was a large production of flowers from June 14 to 29, and this was followed by a series of production peaks until the records were discontinued. The coefficient of correlation of temperature and amount of flowering, when temperature and anthesis for the same day are considered, was −0.21. However, if temperature affects anthesis, some time must elapse between the time that the effect is exerted and the visible effect on flowering. For this reason the coefficients of correlation

of temperature 1, 2, 3, and 4 days before the day of anthesis and the amount of flowering were calculated. The coefficient of correlation of temperature 1 day before anthesis and the amount of flowering was −0.139; of temperature 2 days before anthesis, 0.113; of temperature 3 days before anthesis, 0.499; but for 4 days before anthesis it dropped to 0.435. It therefore appears that there is approximately a 3-day lag between the time that temperature affects anthesis and the time that the effects become visible.

The growing season of 1930 was extremely dry; less than 2 inches of rain fell during the growth of the plants, that is, up to the time the records were discontinued. Flowering may also be dependent upon

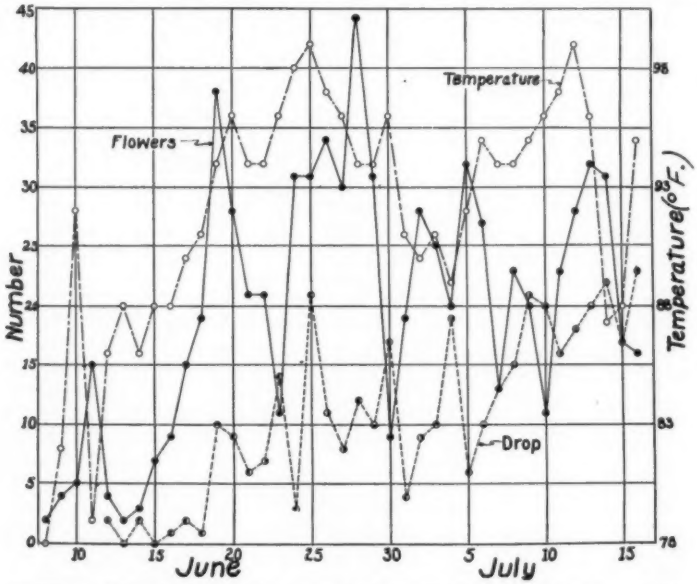


FIGURE 1.—Flowering and flower-drop records for tomatoes during June and July, 1930, with mean temperature. Averages of all plants are shown

rainfall or soil moisture as well as temperature. This may explain the lack of better correlation between temperature and anthesis.

Table 2 and Figure 1 give the number of flowers that dropped each day from the same 10 plants from June 12 to July 16, when the records were discontinued.

An analysis of the data in Figure 1 indicates that the relation of temperature to blossom drop is similar to the relation of temperature to anthesis. The coefficient of correlation of temperature and amount of blossom drop, when temperature and blossom drop for the same day are considered, was 0.413. The value of r when temperature 1 day before blossom drop is considered was 0.476; for 2 days before the drop, 0.499; for 3 days before the drop, 0.68; but for 4 days before the drop the value of r decreased to 0.63. As in the case of temperature and anthesis, these data indicate that there was a 3-day lag between the time that temperature exerted an effect on blossomdrop and the time that the effect became visible.

TABLE 2.—*Blossom-drop data on tomatoes from June 12 to July 16, 1930*

Date	Number of flowers dropped from plant No.										Total	Average
	1	2	3	4	5	6	7	8	9	10		
June 12	1	1									2	0.2
13											0	0
14		1	1								2	.2
15											0	0
16			1								1	.1
17	1	1									2	.2
18	1										1	.1
19	2	1		1	1	1		1		3	10	1.0
20	2	2	1		1		1			2	9	.9
21	1	1		1	1	1	1				6	.6
22		4		1	1	1					7	.7
23	3	7	2		2						14	1.4
24	2		1								3	.3
25		8	2	4	2		2	2		1	21	2.1
26	4	4	1	1	1						11	1.1
27	1	3	1	2				1			8	.8
28		2	2	2		3	1	2			12	1.2
29		2		2			1	3		2	10	1.0
30	1	6		6	1	1	1		1		17	1.7
July 1		1		2				1			4	.4
2	1		2	1		2		1		2	9	.9
3		2	1		1	1	2	2		1	10	1.0
4	4	3	1		1		4	3	2	1	19	1.9
5	2						1		2	1	6	.6
6	2	1	1			1	2	1	2		10	1.0
7			2	1	3	1	2		3	1	13	1.3
8	1	2	2	1	1	1	2	2	2	1	15	1.5
9			2	1	5	3	3	2	3	2	21	2.1
10	2	2	1	2	2	2	3	1	3	2	20	2.0
11			2	1	3	3	2	1	3	1	16	1.6
12	2	1	3	2	2	2	1	2	2	1	18	1.8
13	2	1	2	3	2	1		4	3	2	20	2.0
14	3	3	1	2	3		2	2	3	3	22	2.2
15	2	2	2	1	2	2	1	1	2	2	17	1.7
16	1	4	2	2	3	1	2	2	3	3	23	2.3
Total	41	65	36	39	38	27	34	34	34	31	379	

Observations indicate that the amount of blossom drop was greatly increased by low humidity, hot dry winds, and low soil moisture. This finding agrees with the results obtained by Cross at the Oklahoma station in 1924 and 1925. During the periods of high temperature and low humidity, practically all the styles elongated abnormally. In most instances this elongation occurred before the flowers opened and before the anthers dehisced. (Pl. 1, A.) Thus the stigma and style were exposed to the desiccating winds and they soon became wilted and dried up. Practically 100 per cent of such flowers abort.

ABORTING AND DEVELOPING PISTILS

The external characteristics typically distinguishing the aborting from the developing flowers are a yellowing pedicel and pistil, shriveled blossom, and the development of the abscission layer. Aborting flowers drop naturally or readily fall when jarred or touched.

DEVELOPMENT OF EMBRYO AND ENDOSPERM IN NORMAL PISTILS

The ovules of the tomato are very numerous, completely covering the free surface of the placenta. (Pl. 1, B.) The integument consists of a thick band of tissue closely surrounding the nucellus. It is often difficult to differentiate the integument from the nucellus. As the embryo sac enlarges the cells of the nucellus become much enlarged and elongate perpendicularly to the embryo sac. (Pls. 2, C, and 3, A.)

At anthesis the embryo sac has reached the mature egg stage. The egg is a large densely staining nucleated cell. (Pl. 1, C.) The synergids can be seen at the micropylar end of the embryo sac 12 hours after anthesis. (Pl. 1, D.) Further development of the embryo sac does not take place for more than 82 hours after anthesis. (Pl. 1, E, and pl. 2.) Development of the endosperm after this period is very rapid and is well ahead of the growth of the embryo. (Pl. 3, A, B, C, D.) Within 94 to 130 hours after pollination definite walls have appeared in the endosperm. At 190 hours after anthesis the embryo has reached the stage where differentiation into the dermatogen, periblem, and plerome is just beginning. (Pl. 3, F.) The endosperm has continued to enlarge and lay down separating walls; at 190 hours after anthesis the endosperm has completely filled the embryo sac. (Pl. 3, E.) At 224 hours after anthesis the embryo has grown very rapidly by division of the cells in all four tiers. The division lines between the four groups of cells are very clear. The dermatogen tissue of 1-cell thickness is very conspicuous and comes well down over the sides of the embryo. (Pl. 4, A.) The inner cells of the integument appear to break down, and at this stage the endosperm does not completely occupy the embryo sac. At 237 hours after anthesis the embryo and endosperm are much larger, having grown very rapidly and the periblem initials and suspensor have appeared. (Pl. 4, B.)

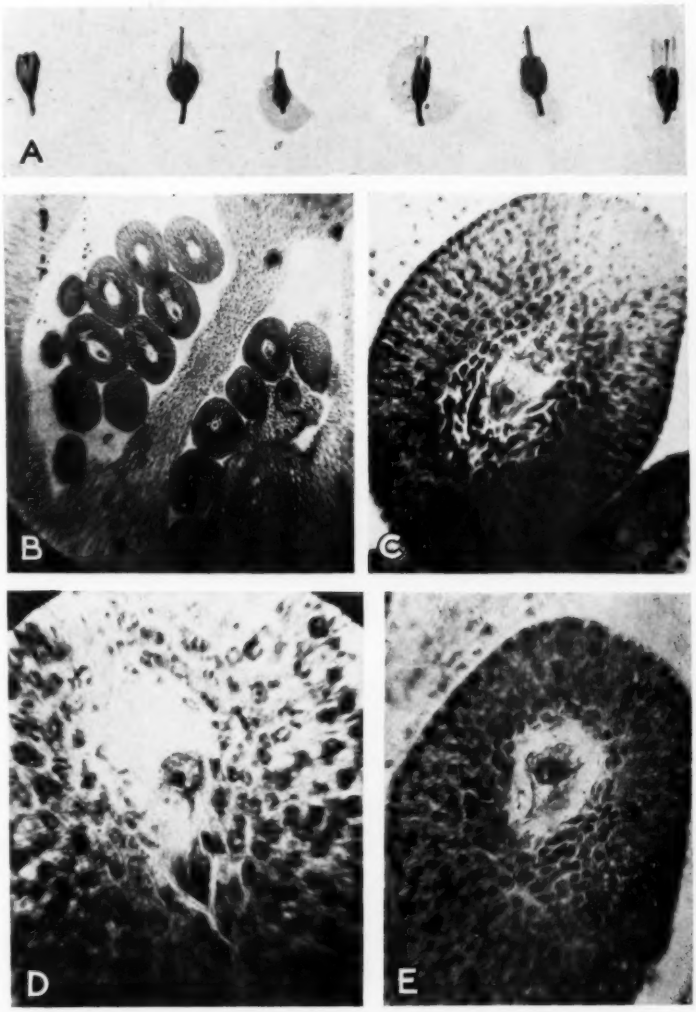
Development of Ovules and Embryo Sacs in Aborting Pistils

The embryo sacs of aborting tomato pistils never developed beyond a weak mature egg stage. The embryo sac and ovules had not developed any farther at 190 hours after anthesis than they had at anthesis. (Pl. 4, C, D, E, F, G.) Other changes in the ovules and embryo sacs are characterized by a light-staining reaction of the cells, very small egg cell and nucleus, and a breaking down of the nucellus and cells of the integuments. The egg cell of the developing pistil is larger than that of the aborting pistil of the same age. (Compare pl. 2, B, with pl. 4, C, and pl. 3, A, with pl. 4, D.) Although the embryo sacs of the aborting pistils have not developed beyond the egg-cell stage at 190 hours after pollination, the embryo sacs of the developing pistils are completely filled with the many-celled endosperm and the embryo has developed at least to the 16-cell stage. (Compare pl. 3, F, with pl. 4, G.)

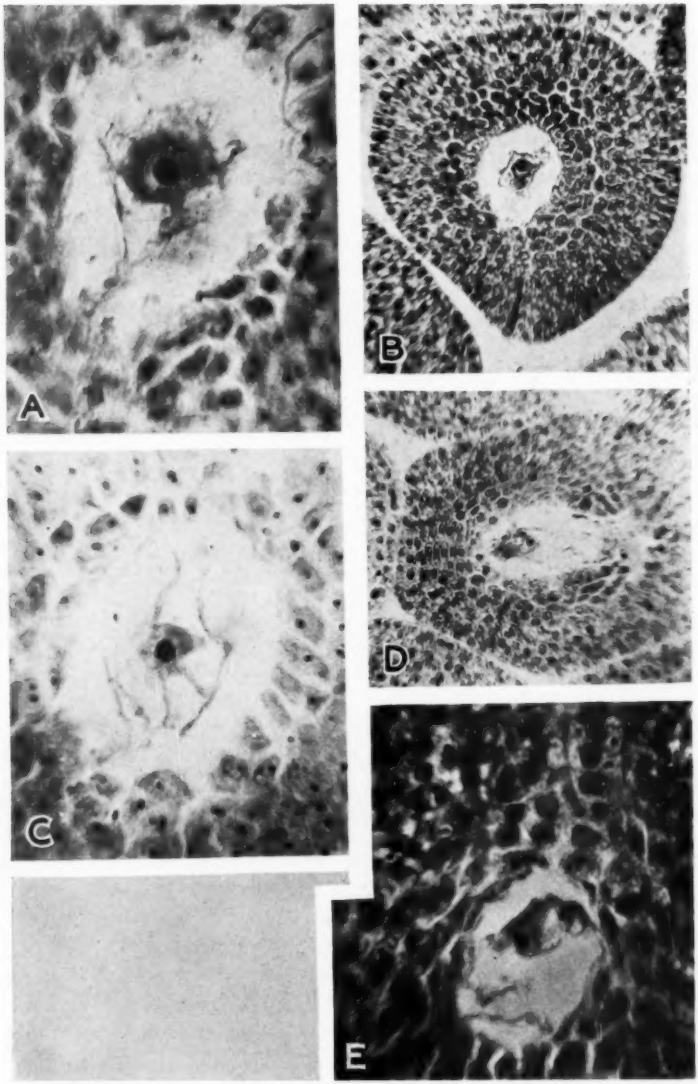
DISCUSSION

The abortion of the pistils studied was not due to lack of pollination, for all flowers were hand-pollinated. Growth of the ovules and embryo sac had ceased at the time of anthesis, when the flowers would normally be pollinated. Although the actual fertilization process was not observed in this study, initiation of growth of the embryo and endosperm was found to occur between 82 and 94 hours after pollination. This was determined by a comparison of the development of the embryo sacs at these two stages. The egg cell of the embryo sac in the aborting pistils was smaller at all times than that of developing pistils.

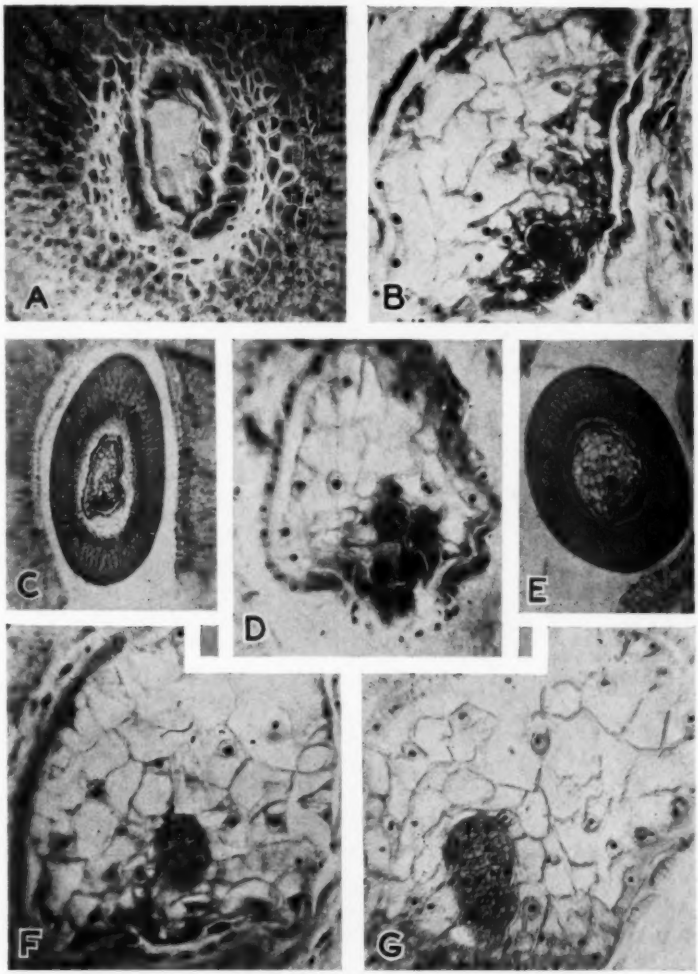
The results here shown do not preclude the contention that lack of pollination and fertilization or unfavorable conditions, such as hot dry winds, may cause pistil abortion and flower dropping. Observa-



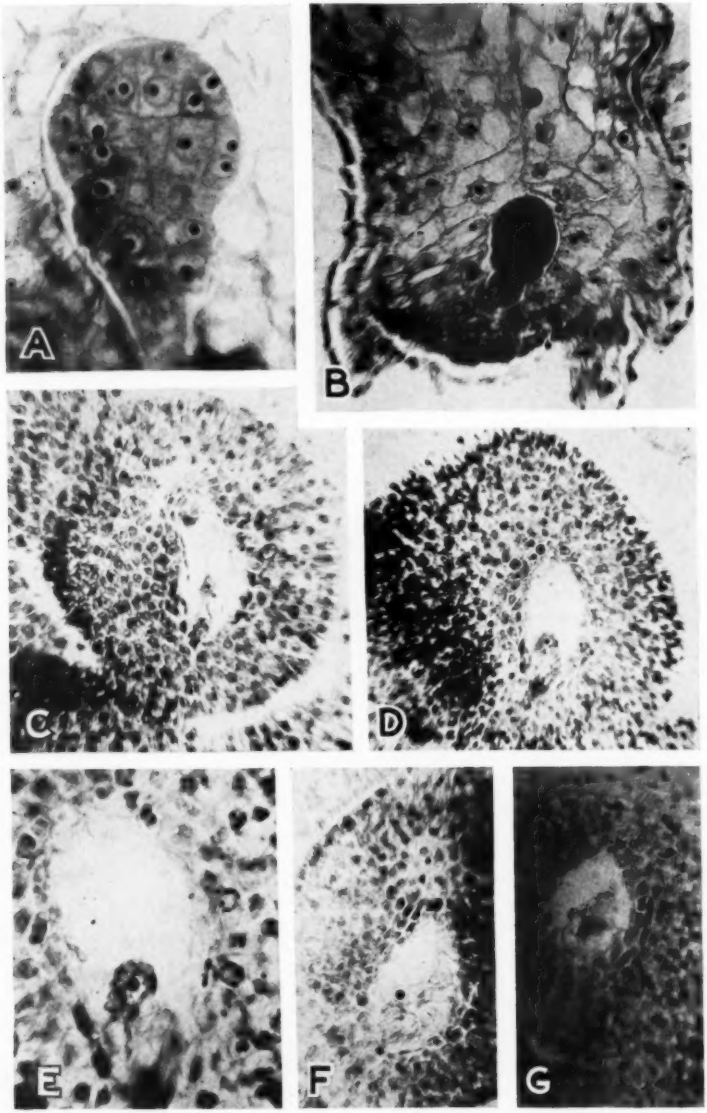
A, Normal tomato flower at the left, all others show abnormally elongated styles previous to anthesis, × ¾; B, portion of pistil, showing development of ovules at anthesis, × 120; C, ovule and embryo sac 2 hours after pollination, × 340; D, 12 hours after pollination, × 650; E, 36 hours after pollination, × 340



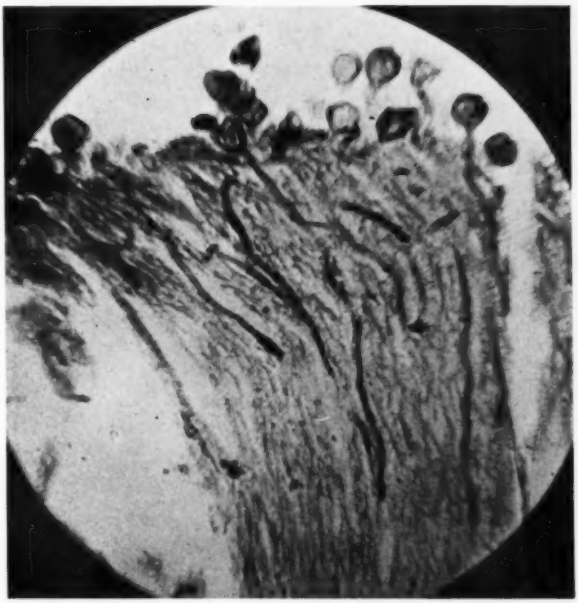
Ovule and embryo sac of the tomato flower at various lengths of time after pollination. A, 36 hours, × 650; B, 54 hours, × 340; C, 60 hours, × 650; D, 70 hours, × 340; E, 82 hours, × 650



Ovule and embryo sac of the tomato flower at various lengths of time after pollination. A, 94 hours and showing first indication of endosperm development, × 340; B, 130 hours and showing embryo development, × 340; C, 142 hours, × 120; D, 151 hours, × 340; E, 190 hours, × 120; F, 190 hours, × 340; G, 224 hours, × 340



A, Ovule and embyro sac of the tomato flower 224 hours after pollination, × 650; B, ovule and embryo sac of the tomato flower 237 hours after pollination, × 340; C, section of ovule of aborted pistil 50 hours after pollination, × 340; D, aborted ovule 94 hours after pollination, ×340; E, the same as D, 94 hours after pollination, × 650; F, the same as D, 106 hours after pollination, × 340; G, the same as D, 190 hours after pollination, × 340



Section of stigma and style showing pollen tube growth 24 hours after pollination, × 340

tions made at Oklahoma by the writer and also by Cross show that, even though pollinated, a great many flowers may drop during unfavorable weather. Plate 1, A, shows the reaction of style growth to low humidity, hot dry winds, and low soil moisture. Only the flower at the extreme left has a style of normal length. These flowers have not yet reached anthesis. Note the dark-colored stigmas which are drying up and collapsing, rendering pollen germination and pollen-tube growth unlikely.

Dorsey,[9] working with potato flowers, concluded that there are physiological influences operating independently of pollen or pistil development which cause the flowers to drop. He found evidences of disintegration in some embryo sacs, but on the whole they appeared to be undergoing the usual growth up to the time they were cut off when the flower dropped. Young [10] states that degeneration changes in the ovules and embryo sacs of potato flowers appear to result from unfavorable environmental conditions and may occur at any stage. In the late megaspore stage, blasting is accompanied by the shriveling of both the megaspore or embryo sac and the cells of the nucellus.

Pollen-tube growth in the tomato appears to be very slow. In Plate 5 it may be seen how short the growth of pollen tubes is 24 hours after pollination. This section shows only one twenty-fifth of the total length of the style. As pollen-tube growth is not initiated immediately after pollination, the actual growth shown in Plate 5 probably occurred in much less than 24 hours. Because of this slow growth of the pollen tube, the stigma, style, and pollen may be destroyed in periods of unfavorable weather before the pollen tubes reach the embryo sac. That this does occur is indicated by the high percentage of abortions observed when weather conditions are unfavorable. The plants from which these flowers were selected were grown in a greenhouse at temperatures ranging from 60° to 75° F. The soil moisture and the humidity of the atmosphere were favorable for good growth and fruit setting. The probable cause of the dropping of these flowers was the low nutrient supply in the soil or the inability of the plant to distribute properly and adequately the elaborated nutrients to the fast-developing embryo.

SUMMARY

The tomato plant has no one definite flowering peak. Flowering seems to be largely dependent upon soil moisture and temperature. The temperature existing approximately three days before anthesis appears to have the greatest influence upon flowering.

Blossom drop is greatly increased by hot dry winds and low humidity as well as by low soil moisture. On 10 plants under observation 47.4 per cent of the flowers aborted. There is a lag of approximately three days between the time that temperature exerts an effect on blossom drop and the time that the effect becomes visible.

During periods of hot dry winds and low soil moisture the styles elongate abnormally, even before anthesis. Few flowers that have elongated styles in hot dry weather develop normally and set fruit.

[9] DORSEY, M. J. A NOTE ON THE DROPPING OF FLOWERS IN THE POTATO. Jour. Heredity 10: 226-228, illus. 1919.

[10] YOUNG, W. J. THE FORMATION AND DEGENERATION OF GERM CELLS IN THE POTATO. Amer. Jour. Bot. 10: 325-335, illus. 1923.

At anthesis the embryo sac of the normal pistil had reached the mature egg cell stage. Under greenhouse conditions no further development of the embryo sac took place for more than 82 hours after anthesis. Initiation of growth of the embryo and endosperm occurred between 82 and 94 hours after pollination. At 190 hours after pollination the embryo had developed to the stage where differentiation into the dermatogen, periblem, and plerome was just beginning.

The embryo sacs of aborting pistils never developed beyond a very weak egg cell stage.

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